From the Department of Clinical Sciences, Danderyd Hospital
Karolinska Institutet, Stockholm, Sweden

DYSREGULATION OF THE IMMUNE SYSTEM IN CHRONIC KIDNEY DISEASE AND THE IMPACT ON DISEASE MANIFESTATIONS AND CO-MORBIDITY

Senka Sendic

Karolinska Institutet

Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8017-103 -8

Dysregulation of the immune system in chronic kidney disease and the impact on disease manifestations and co-morbidity

Thesis for Doctoral Degree (Ph.D.)

By

Senka Sendic

The thesis will be defended in public at Aulan, Danderyd University Hospital, Stockholm, Sweden, on Friday 20th October 2023, at 9am.

Principal Supervisor:
Adjunct professor Joachim Lundahl
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset

Co-supervisor(s):
Professor Stefan H Jacobson
Karolinska Institutet
Department of Clinical Sciences, Danderyd Hospital
Division of Nephrology

Associate Professor Anna Nopp
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset

Opponent:
Senior Professor Bernd Stegmayr
Umeå University
Department of Public Health and Clinical Medicine

Examination Board:
Professor Maria Eriksson Svensson
Uppsala University
Department of Medical Sciences

Associate Professor Guro Gafvelin
Karolinska Institutet
Department of Clinical Neuroscience

Associate Professor Michael Hultström
Uppsala University
Department of Surgical Sciences, Anesthesiology and Intensive Care

"If I have seen further, it is by standing on the shoulders of giants."
−Isaac Newton

To my beloved Family

Populärvetenskaplig sammanfattning

Nedsatt njurfunktion drabbar ca 10 % av befolkningen. Näst efter diabetes och högt blodtryck med nefroskleros (kärlförkalkningar i njurens kapillärer), är olika typer av njurinflammation (så kallad nefrit) de vanligaste anledningarna till njurfunktionsnedsättning. IgA-nefrit (IgAN) är den vanligaste njurinflammationen världen över. En annan vanlig nefrit är lupus nefrit (LN) som kan vara ett delsymtom i sjukdomen Systemisk lupus erythematosus (SLE).

Nedsatt njurfunktion kan medföra flera negativa följdeffekter för individen. En sådan följdeffekt är ökad risk för hjärt- och kärlsjukdomar, som också är den vanligaste dödsorsaken hos njursjuka. En annan konsekvens är försämrad förmåga att bekämpa infektioner. Båda dessa följdeffekter till njursvikt har ett gemensamt ursprung, nämligen förändringar i immunförsvaret. För att förbättra omhändertagandet av njursjuka behöver vi ökad kunskap om de immunologiska förändringar som uppträder vid nedsatt njurfunktion.

Målsättningen med denna avhandling är att skapa en ökad förståelse om de immunologiska förändringar som förekommer vid IgAN samt LN. Vi vill också undersöka markörer i blodet som kan vara inblandade i, eller som kan ge en indikation om framtida kärlförändringarna vid kronisk njursvikt. Slutligen vill vi studera hur inflammationsmarkörer i immunsystemet kan hjälpa oss förstå varför patienter med kronisk njursvikt har en ökad risk att drabbas av svåra infektioner.

Delarbete I Vi studerade 13 patienter med IgAN och jämförde dem med 13 sjukdomskontroller med cystnjuresjukdom och 13 friska kontroller. Vid IgAN produceras förändrat immunoglobulin A (immunoglobulin A förkortas IgA och är en typ av antikropp). Den förändrade IgA uppfattas som kroppsfrämmande vilket leder till produktion av antikroppar mot det förändrade IgA. Detta är en viktig del i sjukdomsutvecklingen vid IgAN. Exakt varför det bildas strukturförändrade IgA-antikroppar vet man inte. B-celler producerar kroppens alla antikroppar, och T-celler medverkar i den processen. Studiedeltagarna lämnade ett blodprov och från det blodet isolerade vi fram B- och T-celler samt monocyter (som alla tillhör kroppens vita blodceller). Vi fann att patienter med IgAN har en förändrad sammansättning av B-celler samt en annan balans mellan B- och T-celler jämför med friska kontroller. Patienterna hade också en högre andel inflammatoriska monocyter.

Delarbete II Vi studerade 89 patienter med SLE, med och utan LN, samt 40 friska kontroller. Vid LN bildas antikroppar som ger sig på kroppens eget DNA. Antikropparna mot DNA brukar vara av s.k. IgG-klass. I denna studie mätte vi istället nivåer av IgE riktade mot kroppseget DNA. Vi visar att patienter med aktiv LN, till skillnad från patienter med inaktiv LN och patienter med SLE utan njurengagemang, har högre halter antikroppar mot DNA av IgE-typ jämfört med friska individer. Vi visar också att faktorer i blodet hos patienter med aktiv LN påverkar granulocyternas, en typ av vita blodceller, uttryck av proteiner och receptorer på cellytan.

Delarbete III Vid studiens start inkluderade vi 103 patienter med kronisk njursvikt. Den gruppen bestod av 54 patienter med mild till måttlig njurfunktionsnedsättning och 49 patienter med kraftigt nedsatt njurfunktion. Vi inkluderade även 54 friska kontroller. Patienterna med kraftigt nedsatt njurfunktion undersöktes endast vid studiens början, medan patienterna med mild till måttlig njurfunktionsnedsättning och friska kontroller följdes under 5 års tid. Studiedeltagarna undersöktes med ultraljud av halsens blodkärl som kan påvisa förekomst av åderförkalkning. Vi mätte också ankel-arm blodrycksindex (ABI). ABI är en kvot mellan blodtryck i armen och blodtryck i ankeln och

kan ge indikation på stelhet i kärlen. Vi använde oss även av en avancerad metod som kallas proteomics. Proteomics möjliggör att man med en liten mängd av patientprov (t.ex. blodprov) samtidigt kan analysera många olika markörer. Vi analyserade 213 markörer i blodet. Därefter valdes tre markörer som på olika sätt kan bidra till kärlförändringar, nämligen sCD14, osteoprotegerin (OPG) och angiogenin (ANG). Dessa tre markörer undersöktes närmare. Vi visar att patienter med kronisk njursvikt har högre nivåer av sCD14, OPG och ANG. Efter 5 års uppföljning är sCD14 och ANG fortfarande högre hos patienter med kronisk njursvikt jämför med friska kontroller. Vi påvisar också ett samband mellan nivåer av sCD14 samt OPG och ankel-arm-index som ett mått på stelhet i artärerna.

Delarbete IV Vi studerade 110 patienter med COVID-19 infektion som vårdades på Danderyds Sjukhus. Som kontroller, utan COVID-19 infektion, ingick 33 patienter med kronisk njursvikt samt 35 friska individer. Vi visar att de patienter med COVID-19 som avled under sjukhusvistelsen, jämfört med de som överlevde, oftare hade nedsatt njurfunktion och hade högre nivåer av MIP-1α och IL-6 (inflammationsmarkörer i immunförsvaret). Vi visar också att nivåer av MCP-1 (en annan inflammationsmarkör) och MIP-1α, oberoende av njurfunktion, är sammankopplat med ökad risk för död på sjukhus till följd av COVID-19.

Popular science summary of the thesis

Chronic kidney disease (CKD) is seen in approximately 10 % of the general population. Next after diabetes and hypertension with nephrosclerosis (hardening of arteries in kidney), different types of inflammation affecting the kidney (entitled glomerulonephritis) are the most common causes of CKD. IgA nephropathy (IgAN) is the most common form of glomerulonephritis globally. Another common nephritis is lupus nephritis (LN) which can affect patients with Systemic lupus erythematosus (SLE).

Impaired kidney function can cause several negative consequences for the individual. One such consequence, is an increased risk of cardiovascular disease which constitutes the most common cause of death in patients with impaired kidney function. Another concern is the reduced ability to fight infections. Both consequences have the same origin, namely alteration in the immune system. An increased knowledge of the immunological changes that occur in patients with declined kidney function is needed, in order to improve the care of patients with CKD.

The aim of this thesis is to gain increased understanding of the immunological alterations in IgAN and LN. We investigate markers present in blood that can promote disease, or give a prognostic indication of future vascular changes in patients with CKD. In addition, we study if inflammatory signaling proteins belonging to immune system can help us to understand why patients with chronic kidney disease have a higher risk of severe infections.

In **study I**, we included 13 patients with IgAN, 13 disease controls (patients with polycystic kidney disease) and 13 healthy controls. In IgAN there is production of aberrant immunoglobulin A (IgA, which is an antibody). The aberrant IgA is recognized as “non-self” by the immune system. This initiates the production of antibodies targeted against aberrant IgA which is an important step in the pathophysiology of IgAN. Why the aberrant IgA is produced is not fully understood. B cells are the source of all antibodies in the body, and T cells are important during B cell maturation. Peripheral blood was drawn from study participants and we isolated B- and T cells and monocytes (another cell belonging to the immune system). We showed that patients with IgAN had an altered composition of B cells subsets and a changed balance between B and T cells compared to healthy controls. In addition, t a higher proportion of inflammatory monocytes was observed in patients with IgAN.

In **study II**, we included 89 patients with SLE, with and without LN, and 40 healthy controls. In LN, antibodies targeted against DNA are produced. These antibodies are usually of IgG isotype. In this study, we measured levels of IgE targeted against DNA. We found that patients with active LN, as compared to healthy individuals, also had higher levels of IgE antibodies against DNA. The other SLE groups did not differ from healthy controls in terms of levels of IgE against DNA. We also demonstrated that serum from SLE patients changed properties of granulocytes, a subpopulation of white cells.

In **study III**, we included 103 patients with CKD, comprising of 54 patients with mild-to-moderate CKD and 49 patients with severe CKD. We also included 54 healthy controls. Patients with severe CKD were only examined at baseline, while patients with mil-to-moderate CKD and healthy controls were followed during 5 years. Study participants were examined with carotid ultrasound as a measure of presence of atherosclerosis. We also measured ankle-brachial index (ABI, the index is a ratio of the ankle blood pressure divided with the blood pressure measured at the arm) as a measure of arterial stiffness. Proteomics is an advanced method, which enables measurement of multiple markers in a very small amount of sample (e.g. blood). We measured 213 markers. Based on the results from

proteomics analyses, three markers, potentially involved in vascular changes, were selected for further investigations: sCD14, angiogenin (ANG) and osteoprotegerin (OPG). We showed, at baseline, elevated levels of sCD14, ANG and OPG in blood in patients with CKD compared to healthy controls. At 5th year of follow up, sCD14 and ANG were still elevated in patients with mild-to-moderate CKD compared to healthy subjects. We also demonstrated that levels of sCD14 and OPG were associated with ABI, as a measure of arterial stiffness.

In **study IV**, we included 110 patients hospitalized due to COVID-19 infection. We also included 33 patients with CKD not infected with COVID-19 and 35 healthy controls. We found that patients with COVID-19 who deceased during hospital stay, more often had declined kidney function and higher levels of MIP-1α and IL-6 (two inflammation markers of the immune system). We also showed that levels of MCP-1 (another inflammation marker) and MIP-1α were associated with increased risk of mortality (independently of kidney function) in hospitalized patients with COVID-19.

Abstract

Background: Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular disease and infections. Both conditions share the same underlying cause comprising of changes in innate and adaptive immunity.

Aim: The objective of this thesis was to contribute to an increased understanding of the dysregulation in the immune system in CKD and to evaluate if selected markers can add prognostic information about the presence of vascular changes and infection outcomes in CKD.

Methods: In **study I,** we phenotyped subsets of monocytes, B and T cells in patients with IgA nephropathy (IgAN) using flow cytometry. We included a disease control group (patients with autosomal dominant polycystic kidney disease (ADPKD)) and healthy controls (HC). Cytokines were analyzed using ELISA. In **study II,** we measured levels of IgE anti double-stranded DNA (dsDNA) in patients with active lupus nephritis (LN), patients with a history of LN, SLE patients with no kidney involvement and population-based controls. Levels of IgE anti-dsDNA were measured using fluorescence enzyme immunoassay. In a subgroup of patients with active LN and healthy controls, we evaluated the effect of active LN serum on healthy donor granulocytes compared to control sera. Granulocytes were stained for markers involved in cell migration, adhesion and immune modulation and were analyzed by flow cytometry. In **study III,** affinity proteomics was used to detect potential biomarkers for early vascular changes in patients with CKD stages 2-3. Three proteins of interest, potentially involved in vascular lesions, were identified and further analyzed with Luminex. Vascular status was evaluated using ankle-brachial index (ABI) and carotid media-intima thickness (CIMT). In **study IV,** we evaluated monocyte related markers in relation to kidney function and mortality in hospitalized patients with COVID-19 infection. As controls we included healthy individuals and patients with CKD without infection.

Results: In **study I,** we demonstrated that patients with IgAN, compared to HC, had an altered balance in B cell subsets, changed balance between B and T cells and an increased proportion of CD19- long-lived plasma cells. Compared to both control groups, patients with IgAN had an increased proportion of non-classical monocytes. We showed an association between sCD40L and MCP-1 levels and urine albumin/creatinine ratio in IgAN. In **study II**, we found higher levels of IgE anti-dsDNA in active LN compared to controls. Other lupus groups did not differ from controls. We also showed that SLE patients with low complement component 3 (C3) levels, as compared to SLE patients with normal C3, had higher levels of IgE anti-dsDNA. We were also able to demonstrate that sera from active LN had a different impact on the phenotype profile of human basophils, neutrophils and eosinophils. In **study III**, comparing the two CKD groups at baseline with healthy controls, higher levels of sCD14, ANG and OPG were observed in both CKD groups. After 5 years, of follow-up, sCD14 and ANG remained higher in CKD stages 2-3 compared to healthy controls. Also, at 5th year of follow-up, a positive correlation was seen between levels of OPG and ABI and between sCD14 and ABI. In **study IV**, we showed that COVID-19 patient who died during hospital stay, as compared to those who survived, were more often in CKD stages 3-5 and had higher levels of IL-6 and MIP-1α. In addition, we demonstrated that levels of MCP-1 and MIP-1α provided additional prognostic information about hospital survival in patients with COVID-19, with either normal or impaired kidney function.

Conclusions: In IgAN, an altered immunological B cell phenotype appears to be mediated by a changed balance between B and T cell subsets, rather than cytokines levels affecting B cell survival, implying the importance of T cell dependent B cell maturation. Determining B cell subsets might be of importance, particularly in terms of number of long-lived B cells, which cannot be targeted through anti-CD20 treatment.

Elevated levels of IgE anti-dsDNA in active LN, but not in other lupus groups, reflect a broader autoreactive mechanism extending the immunological disturbances in LN to also engage IgE-dependent pathways. Serological factors in serum from active LN can moderate granulocyte phenotype implying immunological disturbances in innate immunity during LN flares.

Elevated levels of sCD14 and OPG in patients with CKD stages 2-3 are associated with ABI, as a non-invasive measure of arterial stiffness. This implies the use of sCD14 and OPG as possible biomarkers of vascular lesions even at early stages of CKD. If they can be used as prognostic, or as a possible target for future therapeutic approach warrants further studies.

Patients with COVID-19 who died in the hospital, as compared to patients who survived, were more often in CKD stages 3-5 and had higher levels of MIP-1α and IL-6. Levels of MCP-1 and MIP1α provide additional prognostic information in hospitalized patients with COVID-19, and this is valid both for patients with normal and impaired kidney function.

List of scientific papers

I. **Sendic S***, Mansouri L*, Lundberg S, Nopp A, Jacobson SH, Lundahl J
B cell and monocyte phenotyping: A quick asset to investigate the immune status in patients with IgA Nephropathy *PLoS One. 2021 Mar 19;16(3):e0248056*
* co-authors with equal contribution

II. **Sendic S**, Mansouri L, Svenungsson E, Nopp A, Jacobson SH, Gunnarson I*, Lundahl J*
IgE dsDNA antibody containing serum from SLE patients with nephritis alters the inflammatory profile of basophils, neutrophils and eosinophils. *Submitted for publication.*
* co-authors with equal contribution

III. **Sendic S***, Mansouri L, Hong MG, Schwenk JM, Eriksson MJ, Hylander B, Lundahl J, Jacobson SH
Soluble CD14 and osteoprotegerin associate with ankle-brachial index as a measure of arterial stiffness in patients with mild to moderate chronic kidney disease in a five-year prospective study *Cardiorenal Med. 2023 May 15:1*

IV. **Sendic S**, Mansouri L, Havervall S, Thålin C, Lundahl J, Jacobson SH
Impact of monocyte-related modulators and kidney function on mortality in hospitalized patients with COVID-19 *Scand J Immunol. 2022 Nov;96(5):e13215*

Scientific paper not included in the thesis

Mansouri L, **Sendic S**, Havervall S, Thålin C, Jacobson SH, Lundahl J,
Role of kidney function and concentrations of BAFF, sPD-L1 and sCD25 on mortality in hospitalized patients with COVID-19 *BMC Nephrol. 2022 Sep 2; 23(1):299*

Contents

List of abbreviations

ABI	Ankle-brachial index
ACE-i	Angiotensin-converting enzyme inhibitors
ADPKD	Autosomal dominant polycystic kidney disease
ANCA	Anti-neutrophil cytoplasmic antibody
ANG	Angiogenin
APC	Antigen presenting cell
APRIL	A proliferation inducing ligand
ARB	Angiotensin II receptor blockers
BAFF	B cell activating factor
CD	Cluster of differentiation
CIC	Circulating immune complexes
CIMT	Carotid intima-media thickness
CKD	Chronic kidney disease
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CYC	Cyclophosphamide
C3	Complement component 3
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
ELISA	Enzyme-linked immunosorbent assay
ESKD	End stage kidney disease
Fc	Fragment crystallizable
FEIA	Fluorescence enzyme linked immunoassay
GdIgA1	Galactose deficient IgA1
GN	Glomerulonephritis
HC	Healthy control
Ig	Immunoglobulin
IgAN	IgA nephropathy
IL-6	Interleukin 6
KDIGO	Kidney disease global outcome
LDG	Low-density granulocytes
LN	Lupus nephritis

LPS	Lipopolysaccharide
MAC	Medial arterial calcification
MCP-1	Monocyte chemotactic protein-1/monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
MIP-1α	Macrophage inflammatory protein- 1alpha
NET	Neutrophil extracellular traps
OPG	Osteoprotegerin
PBMCs	Peripheral blood mononuclear cells
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B-ligand
RNA	Ribonucleic acid
Rtx	rituximab
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
sCD14	Soluble cluster of differentiation 14
SCD40L	Soluble cluster of differentiation 40 ligand
SLE	Systemic lupus erythematosus
SLEDAI	SLE disease activity index
TGF-β	Transforming growth factor beta
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alpha
VSMC	Vascular smooth muscle cell
WBC count	white blood cell count
WHO-class	World Health Organization classification

1 INTRODUCTION

1.1 CHRONIC KIDNEY DISEASE

The prevalence of chronic kidney disease (CKD) in the western world has been reported to be approximately 10 %, making declining kidney function a global health problem (1).

The definition of CKD includes glomerular filtration rate (GFR) under 60ml/min per 1.73 m^2 and/or one or more of the following signs of kidney damage: albuminuria (albumin creatinine ratio > 3 mg/mmol) , electrolyte disturbances, urine sediment findings, histological or radiological kidney abnormalities or history of kidney transplantation (2). These abnormalities in kidney structure or function need to be persistent for at least 3 months. The CKD definition was formulated by the Kidney Disease Outcomes Quality Initiative (KDOQI) in 2002 (3, 4).

CKD is categorized five stages (Table 1) according to GFR (stage 1-5).

An estimation of GFR can be done by calculation formulas that use serum creatinine (Cr) or serum Cystatin C (Cys) or both (either by using an average between eGFRcr and eGFRcys or a composite formula which calculates eGFR from both Cr and Cys) (5).

Diabetes mellitus (DM) and hypertension are the leading causes of CKD in high-middle-income countries, followed by glomerulonephritis (6, 7). The most common form of glomerulonephritis in Sweden, as well as globally, is Immunoglobulin A (IgA) nephropathy (IgAN). (8, 9). Another common forms of glomerulonephritis is lupus nephritis (10).

1.1.1 Concerns in CKD

Kidney disease is ranked as the 10th most common cause of death according to Global Health Estimates 2020 (11). Already with a moderate decline in eGFR (eGFR <60 ml/min per 1.73 m^2) an increased overall mortality risk is observed in patients with CKD (12).

It is now widely recognized that CKD constitutes an increased risk for cardiovascular disease independently of traditional cardiovascular risk factors, such as age, smoking, obesity, diabetes mellitus, hypertension and dyslipidemia (12, 13). Death from cardiovascular disease is the most common cause of mortality in patients with GFR <60 ml/min per 1.73 m^2 (12, 14, 15). In addition to the traditional cardiovascular risk factors, CKD is accompanied with additional alterations which are believed to add-on the cardiovascular risk. These non-traditional risk factors include persistent low-grade systemic inflammation, anemia, abnormalities in the calcium and phosphorus metabolism, secondary hyperparathyroidism, endothelial dysfunction and vascular calcification (16).

As kidney function declines, an increased risk of mortality due to infections has been reported (15), being most prominent in patients with advanced kidney failure (i.e. CKD stage 5) (17) . The impaired ability to cope with infections in patients with CKD became evident during the COVID-19 pandemic. Several early reports during the pandemic outlined CKD to be associated with severe complications and an increased risk of hospitalization and mortality due to COVID-19 (18-20).

Table 1. Stages of chronic kidney disease according to international guidelines

GFR stages	Description	GFR ml/min/1.73 m2
1*	Normal or high	≥90
2*	Mildly decreased	60-89
3a	Mildly to moderately decreased	45-59
3b	Moderately to severely decreased	30-44
4	Severely decreased	15-29
5	Kidney failure	< 15

Chronic kidney disease (CKD) stages defined as abnormalities of kidney structure or function during ≥ 3 months. GFR = glomerular filtration rate

*In absence of evidence of kidney damage CKD definition is not fulfilled in stages 1 and 2.

1.2 INNATE AND ADAPTIVE IMMUNITY AND ALTERATIONS OF THE IMMUNE SYSTEM IN CKD

The innate immune system provides the individual with a rapid response when exposed to infection or tissue damage through recognition, phagocytosis, and destruction of pathogens and presentation of antigens on the cell surface. Monocytes, macrophages, dendritic cells, natural killer cells, neutrophils, eosinophils and basophils are cells of the innate immune system (21-23).

The adaptive immune system is composed of B and T lymphocytes and their different subsets. In response to mucosal infections, the naïve B cells undergo maturation and class switching into antibody producing plasma cells, either in a T-cell dependent or T-cell independent manner (24-26) . This part of the immune system can develop a cellular memory after defeating an infection, enabling a more efficient and specific immunological response the next time the same pathogen is encountered (21).

A decline in kidney function affects the immune system in an unfavorably way through an over activation of the innate immune system with a persistent low-grade pro inflammatory state and contemporary alterations of the adaptive immune system, figure 1. Alteration in the innate immunity eventually exposes patients with CKD to an increased risk of cardiovascular diseases and the shift in adaptive immune system affects the ability to cope with infections and the ability to respond to vaccines (22, 27).

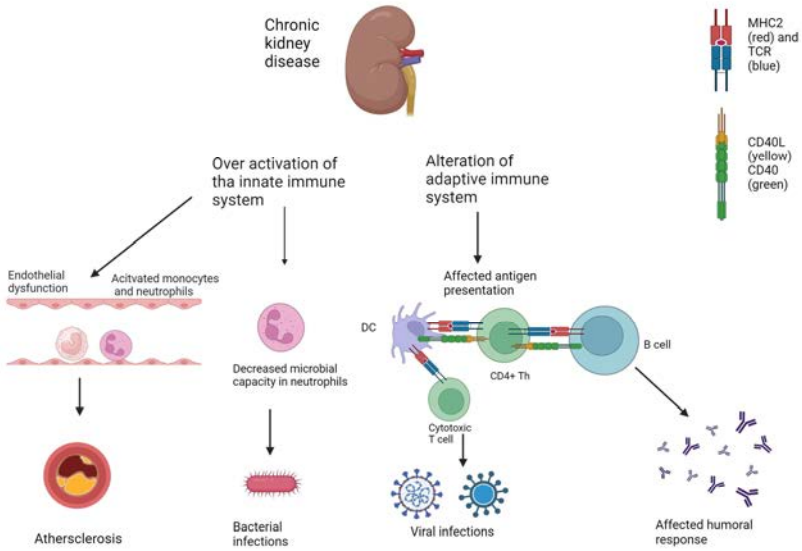


Figure 1. CKD and alterations in innate and adaptive immunity. Over activation of the innate immune system with persistent low-grade inflammation and endothelial dysfunction, activation of neutrophils and monocytes and increased proportion of pro-inflammatory monocytes contribute to atherosclerosis. Decreased microbial ability in neutrophils affects the ability to defend bacterial infections. Dendritic cells (DCs) have a decreased antigen presenting ability, which contributes to reduced capacity to activate T-cells. In addition, T-cells in CKD have a shorter life-span. The affected antigen presentation, affected T cell function and survival, subsequently also affected the humoral response (27, 28). Figure created with BioRender.com

1.2.1 CKD and disturbance in the innate immune system

1.2.1.1 Monocytes

Activated monocytes can produce a subset of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and IL-6 (29) which gives the monocyte a central role in orchestrating the inflammatory response. Monocytes in the peripheral blood express cluster of differentiation (CD) 14 and CD16 on the cell surface. CD14 is the lipopolysaccharide (LPS) receptor and CD16 is the fragment crystalizablle (Fc) gamma receptor III (FcγRIII) (30). According to surface expression of CD14/CD16, monocytes can be phenotyped in three subsets; classical monocytes (CD14++CD16), intermediate monocytes (CD14++CD16+) and non-classical monocytes (CD14+CD16++) (30, 31). Classical monocytes are the most abundant and constitute 90 % of the total monocyte population. The remaining 10 % are intermediate and non-classical monocytes (32). Wound healing and efficient phagocytosis properties are ascribed the classical monocytes (32). The intermediate and non-classical monocytes are regarded to be more pro-inflammatory and increased proportion of intermediate monocytes is reported to be associated with a more rapid kidney function decline (33) and predictive for cardiovascular disease (32, 34) and mortality (35) in patient treated with dialysis.

Several disturbances in monocyte function in CKD have been reported, such as upregulation of pro-inflammatory pathways (36), lower expression of adhesion molecules (37), impaired differentiation to dendritic cells (38), decreased monocyte phagocytosis (21), increased numbers of CD16+ monocytes in the peripheral blood as well as at local inflammatory sites (39). The morphological and functional

monocyte changes in CKD are believed to contribute to an impaired ability to cope with infections (36, 38, 40). In addition, increased monocyte count has been linked to progression of kidney function decline (41) and patients with IgAN display an altered monocyte phenotype, with an increased proportion of non-classical monocytes (42, 43).

1.2.1.2 Basophils

Basophils are rare and comprise less than <1 % of all leukocytes in the peripheral blood (44). Basophils express the high affinity IgE receptor (FcεRI) on their cell surface and are important in the defense against parasites and are key players in allergic disease (45). There is however a growing interest in basophils involvement in several pathways of immune regulation. As an example, secretion of cytokines, such as IL-4 from basophils, promote T helper cell 2 (Th2) differentiation (46). The Th2, basophil and IgE pathway has emerged as an interesting pathway in the pathogenesis of SLE. Patients with SLE can present elevated concentrations of total IgE, not accompanied by allergies, and total IgE levels also correlates with SLE disease activity (47, 48). In addition, SLE patients displayed elevated levels of self-reactive IgE against dsDNA and subsequent formation of circulating immune complexes (CIC) (47). CIC can crosslink IgE bound to the FcεRI on basophils and thereby activate the basophil. Activated basophils are believed to migrate to secondary lymphoid tissue. In the lymphoid tissue, basophils can support survival of antibody producing plasma cells, as well as Th2 formation, resulting in an amplified antibody production in SLE (46, 47). Activated basophils also express B cell activating factor (BAFF, also known as B lymphocyte stimulator, BLyS) and a proliferation inducing ligand (APRIL) important for B cell survival, maturation and class-switch (49).

1.2.1.3 Neutrophils

Neutrophils are important in wound healing. Their ability to phagocyte and release granules containing enzymes, proteolytic proteins and reactive oxygen species provides the neutrophil a central role in the innate immune systems response to bacterial and fungal infections (50).

In CKD, the count of neutrophils in the peripheral circulation is not affected, but neutrophils in patients with CKD have an impaired microbial killing ability (51, 52). In patients with end stage kidney disease (ESKD) and in patients on hemodialysis, the phagocytic ability of neutrophils is affected (53) and in addition hemodialysis procedure appears to affect neutrophil endothelial transmigration (54). Neutrophils is also a cell type of interest in the pathogenesis of SLE. This is mainly through the process of neutrophil extracellular traps (NETs), a neutrophil specific apoptosis mechanism (55). Defects in clearance of apoptotic cells and cell debris are a source for production of autoreactive antibodies against dsDNA in SLE (56). Low-density granulocytes (LDGs) is a distinct neutrophil subset with the ability to produce type I interferon and pro inflammatory cytokines. LDG cells have been proposed to be involved in inducing endothelial damage and inhibiting vascular repair in patients with SLE (57). LDGs also have an increased ability to produce neutrophil extracellular traps (NETs) and LDGs in SLE have a distinct phenotype (58). Mature neutrophils express CD15 on their cell surface (59). There are no LDGs-specific surface markers, but they are regarded to have a more immature phenotype (57, 58).

1.2.1.4 Eosinophils

The biology of eosinophils has most been studied in clearance of helminth infections and in allergic airway inflammation, food allergy and dermatitis (60, 61). In kidney disease, involvement of eosinophils has been described in acute interstitial nephritis, but also in eosinophil granulomatosis with polyangiitis (EPGA), an autoimmune systemic diseases that can affect kidney function (61), and more rarely in SLE (62).

1.2.2 CKD and disturbances in the adaptive immune system

1.2.2.1 T cell changes in CKD

Patients with CKD and ESKD show several changes in their T cell subsets e.g. a lower CD4/CD8 ratio, an elevated Th1/Th2-ratio and decreased levels of naïve and central memory CD4+ and CD8+ cells (63). Naïve and central memory CD4+ and CD8+ cells provide an immunological memory that enhances the response to infections when the same pathogen is reencountered (21, 64). T cells in CKD patients have increased levels of activation markers, resulting in increased apoptosis, rather than T cell proliferation (64). In addition, the consistent low-inflammatory state and uremic milieu in CKD is thought to contribute to the development of exhausted T cells (65). Beside the reported intrinsic changes in T cell function, subsets and cytokine response, there is also reports on affected T cell interaction with antigen presenting cells (APCs) (66).

1.2.2.2 B cell changes in CKD

Several studies have reported decreased levels of B cell subsets in patients with CKD compared to healthy individuals (67-69). B cell lymphopenia is supposedly contributing to the impaired humoral immunity (21) even if there is diversity in levels of immunoglobulins (Ig). Some studies show lower levels of IgG, IgM and IgA (69) while other demonstrate no difference in IgG, IgM and IgA in patients with CKD (22, 66).

B cell targeted therapies, such as the anti-CD 20 antibody rituximab, are used in in systemic diseases affecting kidneys e.g. anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (70) and SLE (71). There are reports on beneficial effect also in IgAN (72) but there is not enough evidence in place to recommend treatment with rituximab in patients with IgAN (73). B cell targeted therapies are of great interest and therefore it is important to understand basics of the maturation process of B cells.

The development of B cells is summarized in figure 2. The process starts in the bone marrow where immature B cells are generated and released into the peripheral blood. During the subsequent maturation process the B cell expresses different cell surface molecules, e.g., CD 19, CD20 and CD38, which vary depending on the stage of B cell maturation (74). In response to an antigen, the naïve B cells undergo maturation and class switching into antibody producing plasmablasts or plasma cells, either in a T cell- dependent or T cell- independent manner (24, 25). The antigen is presented on the T cell receptor and the antigen is recognized by the major histocompatibility complex (MHC) on the naïve B cell. Simultaneously a co-stimulatory signal is generated when CD40 ligand (on T cell) binds to CD40 (on B cell). This leads to cytokine production e.g. TNF-α and IL-10 which stimulates B cell maturation and class-switch from IgM to IgG antibodies (75).

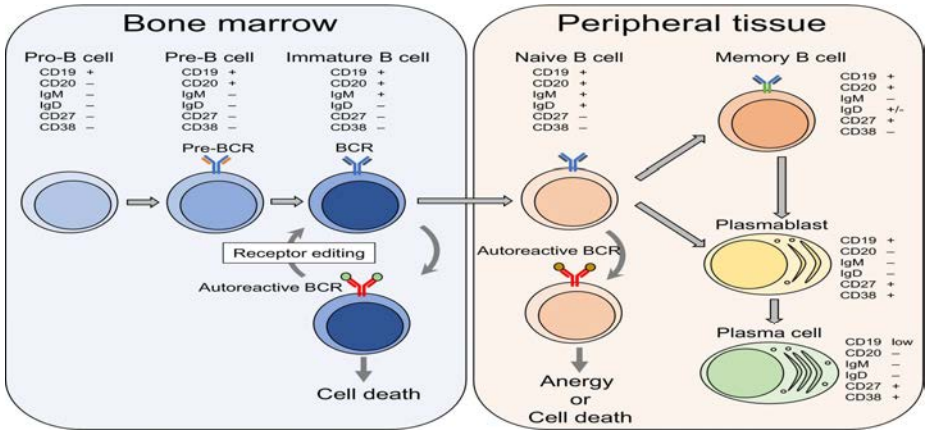


Figure. 2 B cell development.

Figure reprinted from Clinical and Experimental Neuroimmunology, by Noto et al (74). *Used with permission from Wiley Materials.*

1.2.3 Infections in patients with CKD

Infection is the third most common cause of death in patients with CKD (15) and the second most common cause of death in patients with ESKD **(17, 76)**. When adjusted for common risk factors, an increased mortality risk due to infections is also seen in patients with moderate kidney function decline; HR for patients with eGFR < 45 ml/min/1.73 m^2 is 2.36 (95% CI 1.04-5.38) during a median follow-up of 13 years (77). Also, short-term infection mortality is increased in patients with CKD treated for sepsis (78).

The most common infections in patients with CKD are urinary tract infections, pneumonia and sepsis. These account for the majority of infection associated morbidity and mortality (17). Patients with CKD and ESKD are at higher risk for complications (mortality and more severe disease course warranting hospitalization) following seasonal influenza infection (79, 80).

The increased susceptibility to infections, and worse outcomes, in patients with CKD is multifactorial. The uremic milieu favors production of pro-inflammatory cytokines and contributes to impairments in immune responses causing disturbances in both the innate and the adaptive immunity system (22, 28). In addition, iron overload, anemia and elevated intracellular calcium, as part of secondary hyperparathyroidism, are believed to contribute to the disability to cope with infections (51).

1.2.3.1 Coronavirus disease 2019 (COVID-19) and CKD

In December 2019 a novel RNA virus was detected, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2), which affects both the innate and adaptive immune system. The clinical presentation of COIVD-19 varies from mild, asymptomatic infection to respiratory failure with the need of life supporting measures at the Intensive care unit (81). Since 2019 we have come to learn that disease severity in COVID-19 is associated with high age, male gender, obesity, diabetes mellitus, hypertension, pulmonary disease and declined kidney function. Substantial data support that CKD constitutes a major risk for a severe course of COVID-19 and risk of mortality independently of other known risk factors (19, 82, 83). The risk of COVID-19-related death increases gradually as kidney function declines. In an adjusted statistical model including age and other comorbid risk factors, CKD

remains as an independent increased risk for COVID-19 death with a HR 1.33 and HR 2.52 for eGFR 30-60 ml/min/1.72m^2 and eGFR <30 ml/min/1.73m^2 respectively(82).

Reports on over-activation of the immune system displayed as a cytokine storm was reported early during the COVID-19 pandemic. The amplified immune response and inflammatory cytokine production is initiated as the SARS-CoV-2 virus enters the respiratory epithelial cell, inducing an inflammatory response in a downstream pathway mediated by CD14+CD16+ intermediate monocytes and Th1 cells (84, 85). Activated monocytes release CD14 and the soluble form (sCD14) in plasma is regarded as a biomarker of monocyte activation (86). An increased expression of monocyte activation markers sCD14 and sCD163 is reported in patients admitted to hospital due to COVID-19 (87).

Activated monocytes can also release other inflammatory chemokines, and in patients with COVID-19, increased levels of macrophage inflammatory protein 1α (MIP-1α) has been reported (88). MIP-1α, together with monocyte chemotactic protein 1 (MCP-1) acts as a potent chemoattractant to recruit monocytes and other inflammatory cells (89, 90). The severity of COVID-19 is reported to be associated with increased levels of MCP-1 (91).

1.3 IGA NEPHROPATHY

1.3.1 Background and clinical presentation

Ig A nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. Reports on clinical outcomes show that within 10 years of follow-up 10-20 % of patients develop ESKD and after 20 years approximately 40 % of patients have reached ESKD (92-94).

The clinical presentation at diagnosis is diverse. Approximately 1/3 of patients present with an episode of gross hematuria after an upper respiratory infection, another 1/3 of patients are asymptomatic but have microhematuria or mild proteinuria which is detected by occasion when patient's urine is examined with a urine-dipstick. Less than 5% of patients present with acute kidney injury due to crescentic IgA-nephritis, or due to tubular damage caused by red blood cell casts. The remaining patients already have some level of impaired kidney function, proteinuria or hypertension when they are diagnosed with IgAN (9, 93, 95).

To confirm the diagnosis a kidney biopsy needs to be performed. The histopathological diagnosis includes mesangial deposition of IgA1 and in 90 % of biopsies complement 3 (C3) is present. There is also a weaker staining for both IgG and IgM in approximately 50 % of patients (96, 97). The histopathological picture can be classified according to the Oxford Classification system of IgAN, which was first presented in 2009 to facilitate prediction of prognosis and to help to customize and individualize treatment. MEST-C differentiate the kidney injury as mesangial cell proliferation (M), endocapillary hypercellularity (E), segmental sclerosis of glomeruli (S), tubular atrophy and interstitial fibrosis (T), and formation of crescents (C) (98, 99).

1.3.2 Pathophysiology

The multi-hit theory is widely accepted as a model to explain the pathophysiology behind IgA (100-103).

Hit 1: increased production and levels of IgA1 with under galactosylated O-glycans: There are two types of IgA molecules in humans, IgA1 and IgA2. IgA1 is most dominant in the blood circulation, and in IgAN it is also found in circulating immune complexes and in mesangial deposits (25, 102).

On the hinge region of IgA1, glycans are linked to the oxygen molecule of serine/threonine, in a process called O-galactosylation. In IgAN galactosylation is affected and there is an elevated amount of poorly galactosylated IgA1- antibodies, so called galactose deficient IgA1 (GdIgA1). Increased levels of GdIgA1 represents the first hit in disease development (104). The first hit with formation of GdIgA1 is however not enough for IgAN to develop. This hypothesis is supported by studies where relatives of patients with IgAN also present with elevated GdIgA1, though they do not have IgAN (105).

The process of glycosylation defect is not fully understood. Changes in levels of glycosylation enzymes in IgAN compared to healthy individuals have been observed (106). IgA and IgD isotype, and partly IgG3, are the only immunoglobulin isotypes in the human body that undergo glycosylation of the hinge region (107). IgD is produced by mature, but naïve, B cells. IgA1 is produced by B cells which have encountered an antigen, and subsequently undergone class-switch during the later process of B cell maturation. Strikingly, IgD is not under glycosylated in IgAN, possibly indicating that the glycosylation ability is affected during the maturation of B cells (108). This makes the process of B cell maturation highly interesting in IgAN.

Hit 2 and 3: Formation of autoreactive antibodies and formation of immune complexes: The GdIgA1 exposes parts of the hinge region which are normally not exposed, and this causes formation of anti-glycan autoantibodies of IgA1 or IgG type, with a subsequent formation of immune complexes (IC) of GdIgA1-IgG or GdIgA1-IgA1 (101).

Studies show encouraging results in measuring anti-glycan autoantibodies against GdIgA1 as disease biomarkers, although further studies are warranted (109).

Hit 4: Deposition of immune complexes: Deposition of IC in the glomerular mesangium initiates further immunological activation orchestrated by several locally released cytokines such as interleukin 6 (IL-6), tumor necrosis factor (TNF) and transforming growth factor- β (TGFβ). This causes proliferation of mesangial cells, podocyte injury and tubular and glomerular sclerosis (104).

Enzymes involved in glycosylation of IgA1 have been studied and even if differences in levels of glycosylation enzymes in IgAN, compared to healthy individuals, have been observed (106), the process underlying the glycosylation defect is not fully understood. (108). In IgAN the ability to glycosylate seems to be affected during the B cell maturation process.

1.3.3 B cell targeted treatment

The B cell maturation process and the proportion of different B cells subsets in IgAN has gained increased interest, both in relation to the pathophysiology of disease and the possibility for future treatment (110). There are several treatments that affect B cells. Rituximab is a monoclonal anti-CD20 antibody that depletes the antibody producing CD20-positive B cells. This mechanism of action

has beneficial effects in patients with ANCA associated glomerulonephritis and in patients with minimal change disease. In a randomized controlled study, comparing rituximab with standard treatment, rituximab depleted B cells efficiently but the levels of GdIgA and anti-GdIgA antibodies remained at the same level. Moreover, the treatment with rituximab was not more beneficial than standard of care treatment in reducing proteinuria or preserving kidney function. So far, treatment with anti-CD20 antibodies has not shown a clear beneficial effect in IgAN and is not recommended by the Kidney disease global outcomes (KDIGO) (73, 111, 112).

Plasma cells, which do not express CD 20, are not be abolished with rituximab. In contrast BION-1301, an antibody targeting APRIL, affecting maturation and survival of both B cell and plasma cells, managed to reduce levels of GdIgA and proteinuria in IgAN patients (110, 113). Reduced levels of GdIgA1 and proteinuria are recently reported from two Phase 2 trials on fusion proteins (atacicept and telitacicept) with dual effect on both BAFF and APRIL. (114, 115). In addition, an ongoing trial is evaluating the effect of targeting CD38 expressed on plasma cells (CD38+CD20-) in IgAN (IGNAZ; NCT05065970) (110).

1.4 SLE AND LUPUS NEPHRITIS

1.4.1 Background and clinical presentation

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that more commonly affects women. It is a systemic disease that involves several organ systems such as the skin, joints, the brain, lungs and kidneys. To asses general disease activity SLE disease activity index (SLEDAI) can be used. SLEDAI assesses symptoms present at the day of examination or preceding 10 days (116).

Lupus nephritis (LN) is present in almost 50 % of patients with SLE (117). Kidney biopsy is considered as gold standard for diagnosis and histopathological classification of LN. The histologic classification of LN, class 1-6, is done according the 2003 International Society of Nephrology (ISN)/Renal Pathology Society (RPS), (table 2) or World Health Organization Classification (WHO-class) (118). Classification of LN is important in the decision of optimal and individualized treatment (117).

The clinical presentation in patients with LN can range from normal or mildly declined kidney function to a rapid decline in kidney function. Proteinuria is almost always present but can range from low grade proteinuria to the nephrotic range (the latter most common in class V LN, but can also be seen in class III and IV). Patients can also present with microscopic hematuria and red blood cell casts (119), table 2.

Table 2. The histological classification of lupus nephritis

ISN/RPS Class	Histologic Findings	Modifications to Histology	Usual Clinical Findings
1	Normal light microscopy; mesangial immune complexes by immunofluorescence microscopy		None relevant to the kidney so rarely diagnosed or biopsied
2	Mesangial immune complexes/mesangial cell proliferation		Hematuria, low-grade proteinuria; renal insufficiency, nephrotic syndrome not expected
3	Mesangial and subendothelial immune complexes/segmental endocapillary proliferation in <50% of glomeruli	Lesions can be active, chronic, or have elements of both	Hematuria, proteinuria seen in most patients; renal insufficiency, nephrotic syndrome not unusual
4	Mesangial and subendothelial immune complexes/segmental or global endocapillary proliferation in ≥50% of glomeruli	Lesions can be active, chronic, or have elements of both	Hematuria, proteinuria seen in most patients; renal insufficiency, nephrotic syndrome not unusual
5	Numerous subepithelial immune complexes in >50% of glomerular capillaries		Proteinuria, often nephrotic range; hematuria possible; usually no renal insufficiency
6	Glomerulosclerosis in >90% of glomeruli		Renal insufficiency; proteinuria and hematuria often present

ISN/RPS, International Society of Nephrology/Renal Pathology Society.

Reprinted with permission from Clinical Journal of the American Society of Nephrology, Almaani et al (119)

1.4.2 Pathophysiology

Several changes in the immune system contribute to development of SLE, e.g., impaired clearance of apoptotic cells, increased number of autoreactive B- and T cells, which in addition have a decreased activation threshold and production of autoreactive antibodies (120, 121). SLE is characterized by production of several autoreactive antibodies targeted to parts of the cell nucleus i.e., antinuclear antibodies (ANA) which is present in 98% of patient with SLE. Autoantibodies against double-stranded DNA (dsDNA) are the most SLE specific antibodies, present in approximately 60 % of patients with SLE (122). Detailed description of autoantibodies can be found elsewhere (122), herein dsDNA antibodies will be described in more detail since immune complexes containing dsDNA play an important role in LN (120, 122).

When the autoantibodies bind dsDNA, often together with complement (C) factor 1q (C1q) a circulating immune complex (CIC) is formed. CIC accumulate in the kidneys where they may cause tissue damage (123). Autoreactive antibodies against dsDNA are mainly of IgG subtype (75).

There is a growing interest in autoreactive IgE antibodies and basophils in the pathophysiology of SLE. It has been shown that patients with SLE present with elevated levels of IgE anti-dsDNA and that CIC containing autoreactive IgE correlated with disease activity and occurrence of lupus nephritis (124). Also, patients with SLE presented with more active basophils (125). It is suggested that activated basophils in the secondary lymphoid organs can enhance the amplification loop of autoreactive antibody production through interaction with T cells and plasma cells (47, 121). Omalizumab is an anti-IgE treatment approved for asthma and chronic idiopathic urticaria (126, 127). In a safety study of Omalizumab in patients with SLE, SLE patients treated with Omalizumab showed tendency towards decreased disease activity, mainly on rash and arthritis symptoms (128).

1.5 VASCULAR CHANGES IN CKD

1.5.1 Background and clinical presentation

It is now widely recognized that CKD constitutes an increased risk for cardiovascular disease in addition to traditional cardiovascular risk factors, i.e., age, smoking, obesity, diabetes mellitus, hypertension and dyslipidemia. The increased risk of cardiovascular events in patients with CKD is noticed already at an eGFR below 60 ml/min (129).

Besides traditional cardiovascular risk factors, CKD is accompanied with additional alterations which are considered to add-on the cardiovascular risk. These non-traditional risk factors include presence of a persistent low-grade systemic inflammation, anemia, abnormalities in the calcium/phosphorus metabolism, secondary hyperparathyroidism, endothelial dysfunction and vascular calcification (16).

Vascular calcification constitutes an independent risk for CV mortality in patients with CKD (130, 131). Of notice, vascular calcification and arterial stiffness associate with a more rapid loss of kidney function (132, 133).

Atherosclerosis and vascular calcification occur earlier in patients with CKD and have an accelerated course compared with non-CKD patients (129, 134). Vascular changes are reported even in children and young adults with CKD (135). Vascular calcification is observed both in the small arteries of the heart as well as in larger arteries e.g. abdominal and femoral arteries and continue to be present in more peripheral arteries (131). Peripheral artery disease is more common among patients with CKD compared to the general population and the prevalence of peripheral artery disease increases as eGFR declines.(136, 137).

1.5.2 Pathophysiology

Vascular lesions can affect both the intima and media layers of arteries. Lesions affecting the intima layer of arteries are clinically considered as atherosclerotic plaque which can cause lumen obstruction. Lesions affecting the arterial media layer cause calcification within the arterial muscle layer which can be manifested as arterial stiffening (131).

Monocytes, and monocyte-derived macrophages in the endothelial wall, have a central role in the development in the atherosclerotic plaque. Cholesterol is enriched inside the macrophage and presented on their cell surface. When cholesterol is recognized as an antigen by T cells, the immunological reaction of atherosclerosis is initiated (138). Monocytes are also involved in the development of arteriosclerosis and arterial intima media calcifications (139).

During the process of media calcification the vascular smooth muscle cells (VSMC) are believed to turn from a contractile cell to become more osteochondrocytic (140). Transformation from contractile and well-functioning VSMC to osteochondrocytic VCMS is described by three main pathogenic steps) Decreased ability from VSMCs to produce calcification inhibitors such as fetuin A and matrix Gla protein (MGP) b) apoptosis of VSMCs leaving a physical space for remodeling of the media layer and local enrichment of calcium deposits (140, 141). This is considered to be promoted by increased levels of phosphate and calcium-phosphate products in combination with the uremic milieu that exert oxidative stress on VSMCs (140-142).

1.5.2.1 Osteoprotegerin (OPG)

The pathophysiology behind vascular calcification described above highlights the close mechanism of vascular calcification and imbalance in mineral-bone metabolism in CKD (140). Bone resorption starts when receptor activator of nuclear factor kappa-B ligand (RANKL) binds to Receptor activator of nuclear factor kappa-B (RANK). RANK-RANKL binding is inhibited when OPG binds to RANKL which makes OPG an important part of bone remodeling (143). OPG can be produced by several cells e.g. osteoblasts, endothelial cells, vascular smooth muscle cells but interestingly also by neutrophils and B-cells (144, 145).

As a result of endothelial stress and inflammation the endothelial cells release OPG and it is proposed that RANKL/RANK/OPG-axis is central in vascular calcification (144, 146). In a general population, coronary artery calcifications and atherosclerosis in aorta are positively associated with levels of OPG, suggesting a possible role of OPG as a biomarker for atherosclerosis (146, 147). Higher levels of OPG are noted in patients with peripheral artery disease compared to patients with less prominent peripheral artery disease judged by percutan transluminal angioplasty (148). In patients with chronic ischemic heart disease, OPG is associated with coronary artery calcification and cardiovascular mortality after myocardial infarction (149, 150). In type 2 diabetes with microalbuminuria, but asymptomatic of cardiac disease, OPG is an independent predictor of coronary artery disease (151) and predicts cardiovascular and all-cause mortality as well as decline in kidney function in patients with type 1 diabetes (152). There is a positive correlation between increased levels of OPG and vascular calcification in hemodialysis patients (153, 154) and in patients with CKD stages 3-4, levels of OPG were positively associated with 5 year all-cause mortality (155). Further studies are needed to evaluate if the association between OPG and vascular lesions are causative or if OPG levels reflect a protective mechanism to reduce vascular calcification.

1.5.2.2 Angiogenin

Angiogenin (ANG) is a potent angiogenetic protein and has been studied in conditions where the ability of angiogenesis is crucial, such as wound healing and cancer biology (156). Elevated values of ANG have been shown in patients with chronic heart failure as compared to patients with coronary heart disease and healthy individuals (157). Concentrations of ANG increase as kidney function declines (158). The angiogenetic effect of ANG in relation to vascular lesions has been studied, but so far no association with arterial stiffness (158) or peripheral arterial disease and ankle-brachial index has been established (159)

1.5.3 Ankle-brachial index

Ankle-brachial index (ABI) is a convenient and easily accessible first step to evaluate presence of peripheral arterial disease. ABI 0.9-1.3 is considered as normal, and values below and above normal indicate presence of peripheral arterial disease and arterial stiffness respectively (160). A recently published study showed that annual changes (both an increase and a decrease) in ABI of 0.04 in patients with CKD is associated with increased all-cause mortality compared with CKD patients with more stable ABI over a follow up time of 4 years (161). Peripheral arterial disease is more common among patients with CKD compared to the general population and the prevalence of peripheral arterial disease increases as eGFR declines, mainly depending on more patients with ABI >1.4 (136, 137).

1.5.4 Carotid intima-media thickness

Carotid intima-media thickness (CIMT) can be assessed by ultrasound examination. An increased CIMT is regarded as a marker of atherosclerosis (162). A yearly increase of 1 mm in CIMT in patients on dialysis, was associated with a relative risk of 1.29 (1.14-1.47, confidence interval (CI) 95 %) for cardiovascular mortality (162). In patients with CKD a composite risk of fatal and non-fatal cardiovascular disease (RR 1.37, 95 % CI 1.02-1.84) is reported by Karras et al (163).

2 RESEARCH AIMS

The overall aim of this thesis is to contribute to increased understanding of the dysregulations of the immune system in IgA nephropathy and LN and to evaluate if selected markers can add prognostic information about presence of vascular changes and infection outcome in CKD.

The specific aims:

Study I: To evaluate if the immunological phenotype in IgAN is affected judged by; a) proportion of lymphocyte and monocyte subsets, b) balance between B- and T cell subsets and c) levels of inflammatory cytokines and chemoattractants.

Study II: To investigate if levels of IgE anti-dsDNA are elevated in LN and if levels of IgE anti dsDNA can be correlated with disease activity. In addition, to explore if factors in serum from patients with active LN can impact the phenotype of granulocytes.

Study III: To elucidate if potential novel biomarkers can help identify mechanisms and ad prognostic information of presence of early vascular changes in patients with mild-moderate CKD.

Study IV: To investigate if monocyte related modulators can give prognostic information of risk of mortality in relation to kidney function in COVID-19.

3 MATERIAL AND METHODS

3.1 STUDY POPULATION

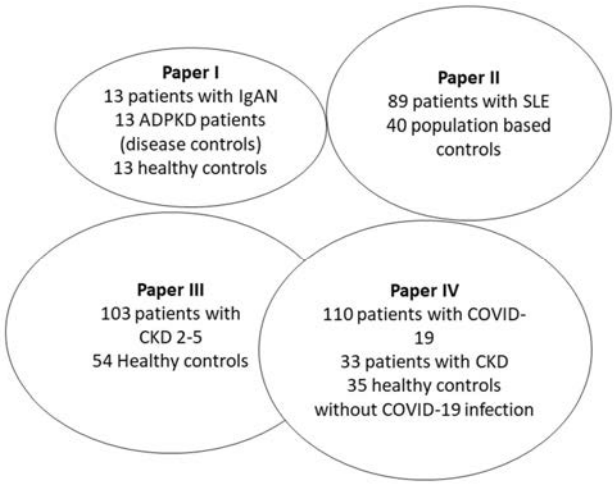


Figure 3. The distribution of study participants in this thesis.

3.1.1 Study I

In this cross-sectional study, we included 13 patients with IgAN and 13 eGFR, sex and age matched disease controls with autosomal dominant polycystic kidney disease (ADPKD). Eligible for inclusion were patients without intercurrent disease and at least eight weeks of stable dose of angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB) prior to sampling. Exclusion criteria were ongoing systemic corticosteroid or immunosuppressive treatment, treatment with corticosteroids within six months prior sampling, diabetes mellitus, cancer, or inflammatory diseases. Patients and disease controls were recruited from the Department of Nephrology, Danderyd University Hospital, Stockholm, Sweden. Thirteen age and sex matched healthy controls were recruited through advertising.

3.1.2 Study II

Paper II is a cross-sectional study including 89 patients with SLE. Thirty patients had active LN with a kidney biopsy within median 2 weeks (IQR 1-4 weeks) from blood and urine sampling. Twenty-two patients had a previous episode of LN, but at the time of sampling no signs of active nephritis and 37 patients had SLE without kidney engagement. These patients are part of a SLE cohort from the Department of Rheumatology, Karolinska University Hospital, Stockholm, Sweden, which has been consecutively including patients during 20 years. All the patients fulfilled at least four of the 1982 American College of Rheumatology criteria. Forty population based controls were included and the only exclusion criteria in the control group was a diagnosis of SLE.

Out of 30 patients with active LN, we selected nine patients with active LN representing a range from low to high titers of IgE anti dsDNA for the cellular analysis. We applied sera from those nine patients

together with sera from 10 healthy controls in cell incubation experiments. Active LN patients were chosen to study the acute impact of serum factors on the phenotype of healthy donor basophils, eosinophils, and neutrophils compared to those of healthy sera (granulocytes isolated from one healthy donor from blood bank, Stockholm, Sweden). Forty population based controls were included as population controls from a larger a larger cohort study, and the only exclusion criteria was a SLE diagnosis (164).

3.1.3 Study III

In this prospective longitudinal study, PROGRESS, 103 patients, aged 18-65 years, with CKD stages 4-5 (n=49) and CKD stages 2-3 (n=54) were recruited from the Department of Nephrology, Karolinska University Hospital, Stockholm, Sweden. Patients with CKD stages 2-3 were prospectively treated, examined and had regular follow-up visits at least two times per year at the out-patient clinic. Patients with CKD stages 4-5 were included as disease controls and were only examined at baseline. Age and sex matched healthy controls were examined and blood samples were drawn at baseline and after five years, with no examination in between. Healthy controls (n=54) were included through advertisement (n=23) and from the Swedish Total Population register (n=31). Healthy controls underwent a written questionnaire to exclude history of kidney disease, cardiovascular disease, diabetes or any ongoing medication. Exclusion criteria for all study participants were known malignancy, kidney transplantation, kidney donation, immunosuppressive therapy or blood-transmitted diseases.

3.1.4 Study IV

During the first pandemic wave in 2020 we included 110 patients with COVID-19 admitted to Danderyd University Hospital, Stockholm, Sweden. Eligible for inclusion were all patients over 18 years old with COVID-19 in whom we had a creatinine value at admission. When blood samples were drawn, 86 % of patients (n=95) were admitted to a general ward, 10 % (n=11) to an intermediate care unit and 4% (n=4) to the intensive care unit.

Thirty-three patients with mild-to moderate CKD, but without COVID-19 infection, were included as a disease control group. They were matched for sex and eGFR, but not matched for age, with COVID-19 patients. We also included 35 healthy subjects who were age- and sex matched with the COVID-19 patients. Both control groups were included from the PROGRESS study (Study III). The rationale for including these two control groups was to compare inflammatory markers in COVID-19 with patients with pre-existing CKD and corresponding eGFR but without infection.

3.2 METHODS

A summary of material and methods is presented in table 3.

Table 3. Overview of material and methods

	Study I	Study II	Study III	Study IV
Study design	Cross-sectional	Cross-sectional	Prospective cohort study	Retrospective cohort study
Number of patients (Total number = patients and controls)	13 (39)	89 (129)	54 (157)	110 (178)
Material	Blood/serum and urine	Blood/serum, urine,	Blood/serum, urine	Blood/serum
Methods	Isolation of fresh PBMCs, flow cytometry, clinical routine biochemical analysis, ELISA	Fresh cell isolation (donor granulocytes), flow cytometry, clinical routine biochemical and serological analysis FEIA	Affinity proteomic, Luminex immunoassay, clinical routine biochemical analysis, ABI, carotid ultrasound	Clinical routine biochemical and microbiological analysis, Luminex immunoassay

PBMCs = peripheral blood mononuclear cells, ELISA = enzyme linked immunosorbent assays FEIA= fluorescence enzyme linked immunoassay, ABI= ankle brachial index.

3.2.1 Blood sampling

- Study I
  For cell analysis: Fresh blood was collected into EDTA tubes, and PBMCs were isolated by density-gradient centrifugation within three hours.
- Study II
  For cell analysis: Fresh blood was drawn into EDTA tubes and the blood was mixed with medium and centrifuged. Supernatant was discarded and blood was washed with medium and centrifuged.
- Study I-IV
  Blood samples were collected, centrifuged and serum (study II and IV) and plasma (study I and III) samples were frozen at -70/-80 ºC for further analysis.

3.2.2 Cell preparation

- Study I
  After isolation of PBMCs a magnetic cell sorting system was used to isolate CD3+ cells (positive isolation) and CD3- (unlabeled cells).
  Thereafter, CD3- cells were used for two panels; B cell panel and monocyte panel for staining for surface markers for flow cytometric analysis.
  The CD3+ cells were subjected to surface marker staining for identification of T cell subsets by flow cytometry.
- Study II

After isolation of granulocytes, cells were destined for two panels: a basophil panel and a neutrophil/eosinophil panel for staining for surface markers for flow cytometric analysis. Cells were incubated with serum from nine patients with active LN and compared to cells incubated with healthy serum. In each panel a negative control was prepared (only blood and medium), and three positive controls comprising of anti-IgE, C5a and *N*-Formylmethionyl leucyl-phenylalanine (fMLP).

3.2.3 Flow cytometry

- Study I – Immunostaining
  To phenotype B cells, the CD3- cells were stained with the following monoclonal antibodies: anti-CD19, anti-CD27, anti-CD38 and anti-IgD.
  To phenotype monocytes, the CD3- cells were stained with anti CD14 and anti-CD16 monoclonal antibodies.
  To phenotype T cell subsets, the CD3+ cells were stained with: anti-CD4, anti-CD8, anti-CD45RA, anti-CCR7, anti-CXCR3, anti-CCR6, anti-CD25, anti-CD127 and anti-CCR4 monoclonal antibodies.
- Study II– Immunostaining
  To study basophils, cells were stained with the following surface markers: anti-CD63, anti-CD203c, anti-CD69, anti-CD164 and anti-CD88.
  To study eosinophils and neutrophils cells were stained accordingly: anti-CD15, anti-CD16, anti-CD44, anti-CD193 and anti-CD88. The biological function of surface markers studied on granulocyte subpopulations is explained below in table 4.
- Study I-II– Flow cytometric analysis
  Following staining, cells were washed and analyzed on flow cytometry within the gates on forward scatter (FSC) and side scatter (SSC) plots. Figure 4 shows an overview of flow cytometry.
  All flow cytometric analyses were performed using the same flow cytometer (Navios, Beckman Coulter, USA).

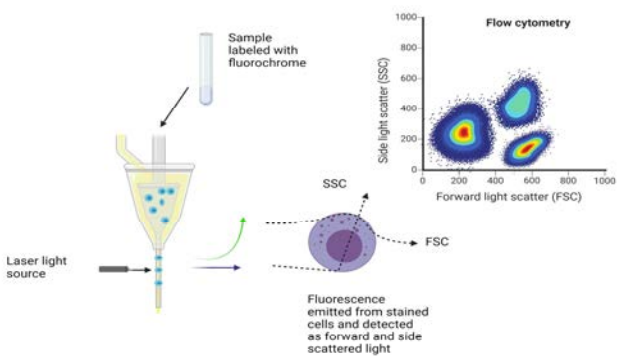


Figure 4. Flow cytometry. Cells are labeled with fluorochromes. Single cells pass through the laser light source. The size of the cell is detected by the light scatter from the forward direction (forward scatter). Light scattered from the side (side scatter) gives information about intracellular granularity. Fluorescence detection adds information about pre-chosen proteins to analyze such as immunostaining with antibodies conjugated with fluorescence (165).

Table 4. Surface markers on granulocytes studied using flow cytometry

Granulocyte population, surface markers	Significance
Basophils	
CD63	Marker of anaphylactic degranulation (166)
CD69	Activation marker (167)
CD88	Complement 5a receptor, chemoattractant. Pro-inflammatory (168, 169)
CD164	Novel activation marker (170)
CD203c	Marker of piecemeal degranulation (166)
Neutrophils	
CD15	Identification marker. Expressed on mature neutrophils, and believed to be involved in cell-cell interactions, phagocytes and is upregulated by degranulation (59)
CD16	Identification marker
CD44	Adhesion and migration marker, important in neutrophil adhesion and rolling and transmigration through endothelium, binds to E-selectin (171)
CD88	Complement 5a receptor, chemoattractant. Pro-inflammatory (168, 169).
Eosinophils	
CD44	Activation and migration marker
CD88	Complement 5a receptor, chemoattractant. Pro-inflammatory. (168, 169).
CD193	Identification marker. Eotaxin receptor CCR3 important for eosinophilic recruitment and migration (172)

3.2.4 Biochemical analysis

C-reactive protein (CRP), creatinine, hemoglobin, white blood cell count, erythrocyte sediment rate, plasma albumin, potassium, phosphate, calcium, IgG anti dsDNA antibodies, IgG, C3, C4 and the urinary albumin/creatinine ratio were analyzed by standard method at the Karolinska University Hospital Laboratory, Stockholm, Sweden according to clinical routine.

Estimated GFR (eGFR) was calculated using the revised Lund-Malmo equation (LM Revised) (study I-II and IV) and Chronic kidney disease epidemiology collaboration (CKD-EPI) equation (study III).

3.2.5 Microbiology analysis (Study IV)

COVID-19 diagnosis was confirmed with nasopharyngeal or oropharyngeal swabs and the reverse transcriptase polymerase chain reaction was performed at Karolinska University Laboratory, Stockholm, Sweden.

3.2.6 Immunoassays for analysis of inflammatory molecules

- Study I
  Plasma from patients with IgAN and healthy controls was distributed on pre-coated enzyme linked immunosorbent assays (ELISA) kits according to manufacturer's instructions to

determine concentrations of following: MCP-1, sCD14, soluble CD40L, BAFF, IL6, Fractalkine and MIP-1α.

- Study II
  Measurement of IgE anti dsDNA levels was performed by EliA which is a fluorescence enzyme linked immunoassay (FEIA). The procedure was done according to manufacturer's instructions and the plates were analyzed with Phadia250 instrument.
- Study III
  Levels of sCD14, ANG and OPG were measured using Luminex Discovery Assays according to manufacturer´s instructions.
- Study IV
  Levels of MCP-1, MIP-1α, IL-6 and sCD14 were measured using premixed magnetic Luminex assays according to manufacturer´s instructions. The plates were analyzed with Bio-plex MAGPIX Multiplex reader (BioRad).

  Luminex is a convenient method since it enables analyses of multiple analytes at the same time, using small amount of sample. The method uses spherical pre-colored beads to capture specific antigens and the levels of analytes were analyzed using fluorescence for detection (173).

3.2.7 Affinity proteomics (Study III)

Prior to carrying through the affinity proteomic a thorough literature review was performed to define potential biomarker candidates in CKD. Through search on The Human Protein Atlas(174) and PubMed, the candidate proteins were compiled into five groups according to their biological and potential pathophysiological role in CKD; *inflammatory markers*, proteins involved in *fibrosis*, *kidney disease marker*, *cardiovascular* marker and proteins involved in *bone metabolism*. This rendered in a list of 213 candidate proteins.

Affinity proteomics enables the analysis of multiple proteins at the same time using a small amount of patient material (in Study IV plasma was used. The method is previously described in detail (175, 176). Briefly, antibodies, which target the protein of interest, are coupled with magnetic and color-coded beads. Patient's samples were diluted and prepared by labelling with biotin. Following that the prepared patient´s samples were incubated with the beads. Next fluorescent streptavidin was added, and the fluorescent levels (per bead population) were analyzed in a Luminex FlexMap3D cytometer (figure 5). The affinity proteomics was performed at the Science for Life Laboratory, Stockholm, Sweden.

Patients with CKD stages 4-5 were included as disease controls and proteomics was only performed at baseline Affinity proteomics at baseline and after 5 years follow-up was done for patients with CKD stages 2-3 and healthy controls. After comparisons of significantly different proteins at baseline and after 5 years, we decided to focus on three proteins involved in occurrence of vascular lesions: osteoprotegerin (OPG), anigogenin (ANG) and soluble CD14 (sCD14). These three proteins were quantified with Luminex Discovery Assays as described in section above 3.2.6.

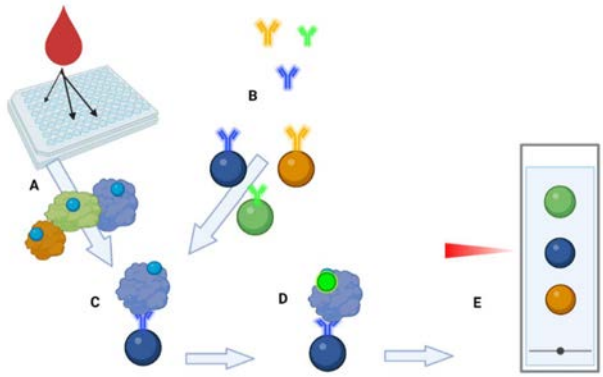


Figure 5.Affinity proteomics. Samples from patients and controls are diluted and placed on 96-well plates. A) Proteins are labeled with biotin, B) antibodies and color-coded beads are coupled. C) Antibody bind their target protein and streptavidin conjugated fluorophore are added and act as reporter solution. E) The fluorescent levels (per bead population) is analyzed in a Luminex FlexMap3D cytometer (177).

3.2.8 Clinical investigations (Study III)

- Ankle-brachial index (ABI)
  After 5-10 minutes resting blood pressure (BP) was measured two times on left upper arm and two times on right upper arm. Thereafter the BP cuff was applied around the ankle and the systolic blood pressure (SBP) was measured, twice on the left ankle and twice on the right ankle, in the posterior artery or dorsalis pedis artery using a doppler stethoscope. ABI was calculated as the ratio between SBP in the leg and SBP in the arm for the left and right side respectively. This resulted in four ratios and the lowest ratio was selected.
- Carotid ultrasound
  Carotid intima-media thickness (CIMT) was measured on the common carotid artery approximately one cm proximal of the carotid bulb. Measurements were done on both the left and right side, and the mean value was used. Examinations were performed by experienced sonographists at baseline and after five years follow-up using an ultrasound device from Sequia 512, Siemens Medical Solutions, Mounatin View, CA, USA.

3.3 STATISTICAL ANALYSIS

Demographics and base line characteristic are presented as numbers and percentages when applicable. Data was checked for skewness and normal distribution was checked using the Shapiro Wilks test and quantile-quantile (QQ) plot. For normally distributed values differences between the groups were calculated using one-way analysis of variance (ANOVA) since there were three or more groups to compare. For data that did not fulfill the normality distribution, differences between groups were calculated using a non-parametric tests and values are presented as medians and interquartile range (25-75%). When comparing two independent groups the Mann-Whitney U test was used. The Kruskal-Wallis test was used to compare three or more independent groups. Post-hoc analysis following Kruskal-Wallis was calculated with Dunn´s test. The Bonferroni correction was applied to control for multiple comparisons. For paired samples Wilcoxon matched-paired signed rank test was used. For categorical variables the Chi-Square and Fischer´s exact tests were used. To analyze correlations between variables Spearman´s rank correlation and linear regression was used. Risk of mortality (study IV) was analyzed using multiple logistic regression using odds ratio.

A p-value <0.05 was considered statistically significant.

3.4 ETHICAL CONSIDERATIONS

All studies in this thesis were performed according to the Declaration of Helsinki (178). There are several ethical principles to align with.

Two ethical principles are voluntary participation and informed consent. All study participants were given study oral and written information and had time to think about the information before their decision to participate in the study. Study participants were also informed that they participate voluntary and that they can withdraw their participation at any time without the need to explain why and with no negative consequences for them. All study participants in these four studies provided written informed consent.

Another ethical consideration is the aspect of personal integrity. This is very important and was addressed by several means. In study one, where patient inclusion was done by doctor Senka Sendic (SS), the code key was stored in a separate and secure place only accessed by me. Medical data used was connected to coded identities and only project members had access to this information. In study II-IV we only had access to coded patient data and the patient identity was unknown to us. Only the principal investigator of each project had access to the code key.

When conducting research it is most important to do no harm. Regarding physical harm, vein puncture for blood sampling is for most people a minor discomfort, with almost no medical risk and we consider it to be ethical with respect to the potential gain in regard of research findings and potential future benefits for patients. In addition, in study I, the blood sample for research purpose was obtained at the same time as the patient was scheduled for blood sampling for clinical purpose. In study II, patients were examined with clinical examinations (ultrasound examinations, ankle-brachial index), meaning the study participants were obligated to spend extra time to undergo examinations and as patients with CKD already spend much time in the health care system this was addressed by careful planning so that most could be done at the same occasion.

4 RESULTS AND DISCUSSION

4.1 STUDY I

4.1.1 Result and discussion

Study population

We included 13 patients with IgAN, and two control groups, one comprising of 13 healthy individuals (HC) and one disease control group of 13 patients with ADPKD. All groups were matched for sex and age. There were no significant differences in age (p=0.894) or eGFR (p=0.092) between the three groups. There was no significant difference in blood pressure or use of ACEi/ARB between IgAN patients and the ADPKD group. Urine albumin/creatinine-ratio was higher and plasma albumin was lower In patients with IgAN compared to ADPKD patients (p=0.001 and p=0.015 respectively). Baseline characteristics are shown in table 5 and baseline laboratory data are shown in table 6.

B subsets

We identified the following B cell subsets; naïve B cells, pre-switched B cells, switched B cells, plasma blasts and long-lived plasma cells in peripheral blood, using four cell surface markers (CD19, CD27, CD 38 and IgD) (table 7).

Patients with IgAN had lower proportions of pre-switched B cells and plasma blasts compared to HC (p=0.005 and p=0.01 respectively) but a higher proportion of long-lived plasma cells (p=0.002) (figure 6). We did not observe a significant difference between patients with IgAN and ADPKD in terms of B cell subsets.

Patients with IgAN displayed a higher ratio between proportion of naïve and pre-switched B cells compared to patients with ADPKD and HC (p=0.02 and p=0.02 respectively) (figure 7). A higher ratio between proportion of naïve/pre-switched B cells in IgAN as compared to both control groups indicates an altered balance in the peripheral blood B cell subsets in patients with IgAN. This may mirror a relocation of pre-switched B cells from peripheral blood in patients with IgAN. There is increasing evidence reported by others, supporting the theory of migration of B cells from the peripheral blood to mucosa associated lymphoid tissue where they are primed to produce GdIgA (179-181).

T cell subsets and relation between Th2 and B cell subsets.

The following T-cell subsets were identified; CD4+naïve, CD4+ memory, CD8+naïve and CD8+ memory, Th1, Th2, Th17 and regulatory T cells. No difference in terms of T cells subsets was detected between the three study groups (data not shown). We demonstrated a lower ratio between plasmablasts and Th2 ($p<0.001$, figure 8) and between pre-switched B cells and Th2 ($p<0.001$, figure 8) in IgAN compared to HC.

T cells are regarded as important in the pathogenesis of IgAN, and increased proportions of circulatory Th2 compared to Th1 has been reported in IgAN in several studies (182) accompanied by elevated levels of Th2 cytokines IL4, IL6 and IL-13 (183).

Monocyte subsets

Based on the surface expression of CD14 and CD16 we identified the following monocyte subsets; classical (CD14++CD16-), intermediate (CD14++CD16+) and non-classical (CD14+CD16++). Patients with IgAN showed an increased proportion of non-classical monocytes compared to both control groups ($p=0.004$ and $p<0.001$ respectively), figure 9. The expansion of non-classical monocytes in the peripheral blood in IgAN is in line with previous studies (42). Non-classical monocytes are considered as pro-inflammatory in both acute and chronic inflammation, in contrast to classical monocytes which are more phagocytic rather than inflammatory (184).

sCD40L and BAFF

To increase the understanding of B cell maturation, class-switch and B cell survival we measured BAFF and sCD40L in IgAN patients and healthy controls.

No significant difference in levels of BAFF and sCD40L were detected between the two groups. However, we show a negative correlation ($R^2=0.32$ $p=0.004$) between levels sCD40L and proportions of pre-switched B cells (figure 10). CD40, expressed on B cells, binds to CD40L expressed on surface of activated T cells (185). This cell interaction is important in the T cell dependent maturation and class-switch of B cells. Our finding suggests an interference of sCD40L in the T-cell dependent B cell maturation and class-switch. Soluble form of CD40L can bind to CD40 and thereby prevent CD40-CD40L interaction between B and T cells, which has previously been described in patients receiving hemodialysis as well as in LN (186, 187). In line with this, is our finding of higher proportions of pre-switched B cells in patients with IgAN. No difference in switched B cells was seen in IgAN compared to controls.

Next, we wanted to explore sCD40L from a clinical perspective. The level of sCD40L was associated with increased UACR in patients with IgAN ($p<0.001$) Figure 10. Recently one published study showed a negative association between levels of sCD40L and UPCR, with decreasing levels of sCD40L when UPCR increases (188). Further studies are needed to understand the relationship between UACR and sCD40L in IgAN.

IL-6 levels and relationship to UACR and plasmablasts

IL-6 levels in the peripheral blood were significantly higher in patients with IgAN ($p=0.03$) compared to healthy controls (Figure 11). Also, we report a negative association between IL-6 levels and plasmablasts in the peripheral circulation ($R^2= 0.39$, $P=0.03$) (Figure 11).

IL-6 is a pluripotent cytokine with the ability to shift the Th1/Th2 balance in favor of Th2, promoting Th2 mediated B cell survival, activation and antibody production (189). IL-6 is both acting autocrine and paracrine (190), and although plasmablast need IL-6 for further maturation, the plasma blast itself can also produce IL-6 (191). Plasmablasts found in the peripheral circulation are regarded as migratory, moving between lymph nodes, the spleen and bone marrow (192). If high levels of IL-6, together with low lower levels of plasmablasts in the present study, reflects the ability to enhance plasmablast exit from the circulation needs further research.

Monocyte related cytokines and relationship to UACR

No significant differences were detected in levels of MCP-1, MIP-1α, sCD14 or fractalkine between patients with IgAN and healthy controls. We demonstrate a linear relationship between MCP-1 and UACR in IgAN, (R^2=0.34, p= 0.03) (Figure 12). MCP-1 has previously been shown to associate with both tubular injuries, and glomerular diabetic nephropathy (193-195).

Table 5. Baseline characteristics of study participants

	Demographic characteristics of study objects					
	IgAN		ADPKD[1]		HC[2]	
	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)
Age (years)	45 (38–60)		42 (37–56)		44 (37–63)	
Gender (men)		6 (46.2%)		6 (46.2%)		6 (46.2%)
eGFR[3] (ml/min/1.73m^2)	57 (42–84)		57 (36–81)		81 (72–86)	
SBP[4] (mmHg)	125 (112–136)		128 (120–142)		N/A	
DBP[5] (mmHg)	80 (77–85)		83 (75–88)		N/A	
ACEi[6] or ARB[7]		8 (61.5%)		9 (69.2%)	N/A	
Lipid lowering drugs		6 (46.2%)		4 (30.8%)	N/A	

[1]ADPKD: autosomal dominant polycystic kidney disease (disease controls),
[2]HC: Healthy controls,
[3]eGFR: estimated glomerular filtration rate,
[4]SBP: systolic blood pressure,
[5]DBP: diastolic blood pressure,
[6]ACEi: angiotensin converting enzyme inhibitor (enalapril),
[7]ARB: angiotensin receptor blocker (candesartan or valsartan),
N/A: not applicable. Values are given as median and interquartile range (25–75%).

https://doi.org/10.1371/journal.pone.0248056.t001

Table 6. Laboratory findings

	Laboratory Findings		
	IgAN	ADPKD	P-value*
	Median (IQR)	Median (IQR)	
Creatinine (μmol/L)	88 (73–150)	99 (75–161)	0.626
UACR[1] (mg/mmol)	74 (17.5–115.5)	1.6 (1–6.2)	**0.001***
CRP[2] (mg/L)	1 (1–1.5)	1 (1–4.5)	0.336
Hb[3] (g/L)	136 (129–145)	129 (123–141)	0.248
WBC[4] (10^9/L)	5.9 (4.6–6.5)	6,2 (4.8–8.1)	0.293
P-Alb[5] (g/L)	34 (31.5–37.0)	37 (35.5–40.0)	**0.017***

[1]UACR: urine albumin creatinine ratio,
[2]CRP: c-reactive protein,
[3]Hb: Hemoglobin,
[4]WBC: white blood cell count,
[5]Alb: Albumin. Values are given as median and interquartile range (25–75%).
* Significant P-value, using Mann- Whitney U test.

https://doi.org/10.1371/journal.pone.0248056.t002

Table 7. Identified B cell subsets

Identified B cell subsets	Cell surface identification marker
Naïve B cells	CD 19+ CD27-, IgD+
Pre-switched B cells	CD 19+ CD27+, IgD+
Switched B cells	CD 19+ CD27+, IgD-
Plasma blasts	CD 19+ CD27+ CD38+
Long-lived plasma cells	CD 19- CD27^{hi} CD38^{hi}

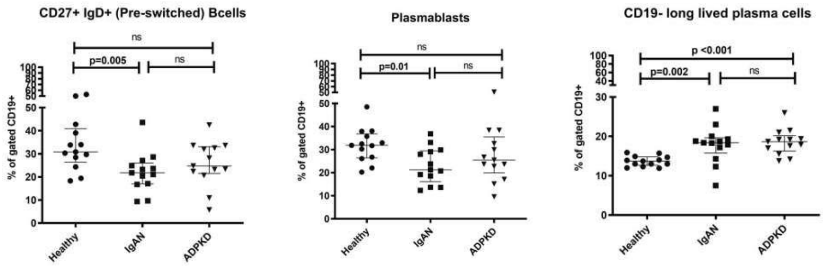


Figure 6. Proportion of pre-switched B cells, plasmablasts and long-lived plasma cells B cells. Comparison between the groups was done using the Kruskal-Wallis test. Scatter plots represent the range with whiskers and the median as the middle line.

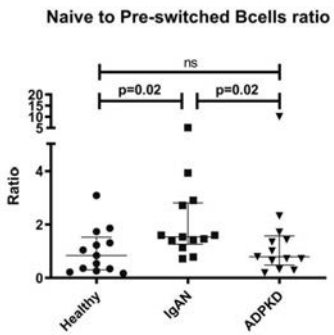


Figure 7. Ratio of naïve/pre-switched B cells in healthy controls, IgA nephropathy patients and patients with autosomal dominant polycystic kidney disease (ADPKD). Comparison for cell fractions were performed using the Kruskal-Wallis test. Scatter plots represent the range with whiskers and the median as the middle line.

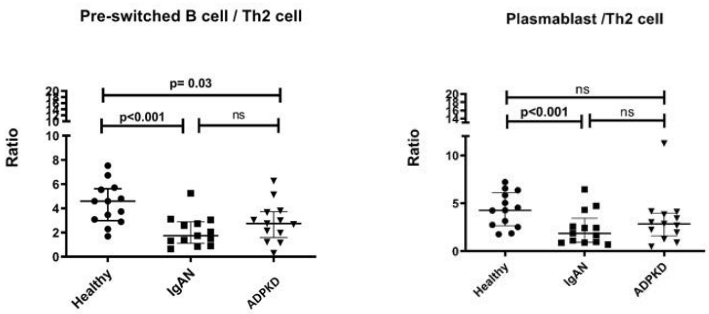


Figure 8. Ratio of proportions of pre-switched B cell to Th2 cell and plasmablast to Th2 cells. Comparison for cell fractions were performed using the Kruskal-Wallis test, P<0.005 was considered significant. Scatter plots represent the range with whiskers and the median as the middle line.

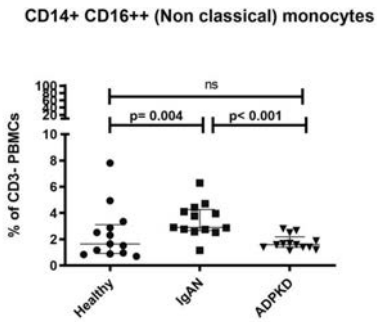


Figure 9. Proportion non-classical monocytes in patients with IgAN, ADPKD and healthy. Comparisons for cell fraction were performed using the Kruskal-Wallis test, P<0.005 was considered significant. Scatter plots represent the range with whiskers and the median as the middle line.

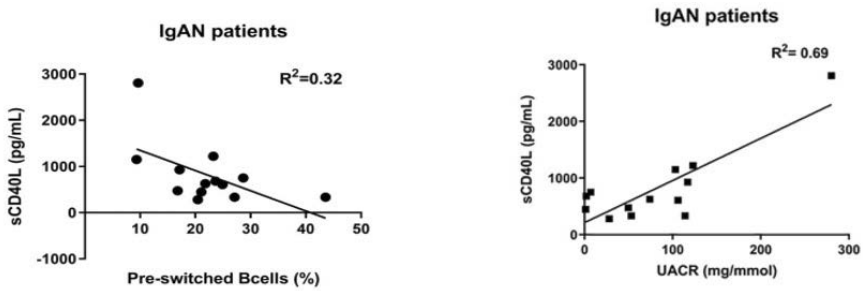


Figure 10. sCD40L relationship to A) pre-switched B cells R^2=0.32 and p=0.03 and B) urine albumin creatinine ratio (UACR) R2=0.69 and p< 0.001 using linear regression test.

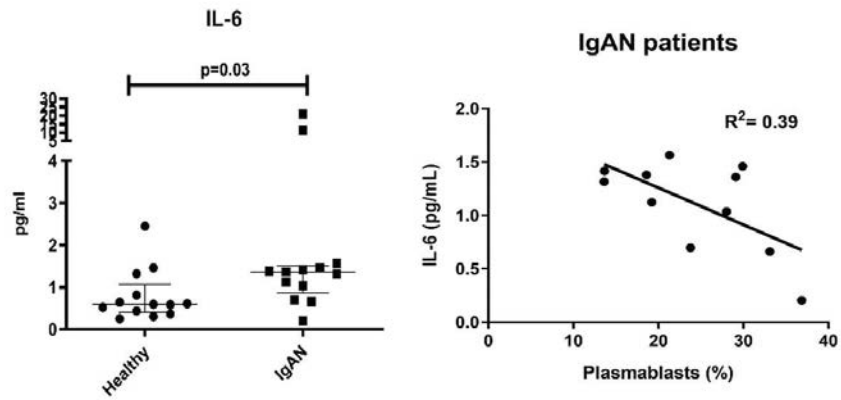


Figure 11. IL-6 levels and correlation to plasmablasts in IgAN. Higher level of IL-6 in patients with IgA nephropathy comparing to healthy controls p=0.03 (comparison was done using the Mann-Whitney U test) Relationship between IL6 levels and plasmablast, p=0.03 (Analyzed with linear regression test).

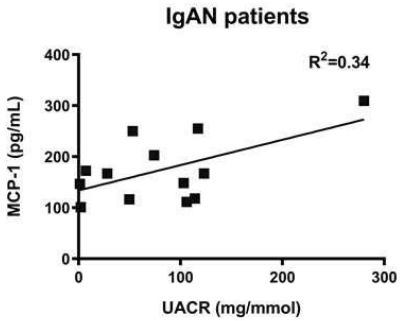


Figure 12. The relationship between MCP-1 and urine albumin/creatinine ratio in patients with IgAN using a linear regression test.

To summarize, demonstrated that patients with IgAN display alterations in B cells subsets in peripheral blood, with a lower proportion of pre-switched B cells and plasmablasts but an increased proportion of CD19- long-lived plasma cells.

We also found a lower ratio between plasmablasts/Th2 and pre-switched B cells/Th2 in IgAN compared to healthy. This indicates a changed balance between B cell subsets and Th2 in patients with IgAN. This is also supported by our finding of a negative correlation between sCD40L and pre-switched B cells. In addition, we showed a positive correlation between sCD40L and urine albumin/creatinine ratio in IgAN.

In patients with IgAN, the level of MCP-1 was positively correlated with urine albumin/creatinine ratio, and patients with IgAN have an increased proportion of non-classical monocytes, implying involvement of non-classical monocytes in the disease process.

4.1.2 Limitations

There are several limitations in our study. One important limitation is lack of sample size calculations and the small number of patients which can limit the possibility to detect difference in cell subsets or cytokine levels between study groups and controls.

There are methodological considerations. There is lack of a standard staining procedure to identify subsets of B and T cells which makes it more difficult to compare results between studies (179, 196). Hence, different cell staining approaches might affect the proportion of T and B cell subsets. We attempted to address this by using a well-defined immune phenotyping approach (197) with a limited numbers of cell surface markers which would make phenotyping clinically more accessible.

During cell preparation, we used a magnetic cell sorting system to isolate C3+ cells from C3- cells. There is a risk that the magnetic sorting can affect fragile cells and hence interfere with the results in terms of proportion of identified cell subsets. Evaluation of magnetic cell sorting seems however to be comparable with flow cytometry (13). The laboratory sorting procedure was identical for cell separation in patients with IgAN and the two control groups, and if cells from IgAN were not more fragile, we can assume the magnetic sorting is supposed to affect samples equally.

4.2 STUDY II

4.2.1 Results and discussion

Study population and baseline characteristics, immunosuppressive treatment and laboratory values

We included 89 patients with SLE and 40 population-based controls (hence referred to as controls). Patients were divided into three groups: 30 patients with active LN, 22 patients with history of LN and 37 patients without renal involvement. There was no significant difference between SLE patients and controls in terms of age, ethnicity and blood pressure control. Patients with active LN, in contrast to patients with history of LN and SLE patients without kidney involvement, were more likely to have higher dose of prednisolone and to be treated with cyclophosphamide, mycophenolate mofetil and rituximab. Baseline characteristics of study participants is presented in table 8.

Patients with active LN had significantly higher UACR, lower plasma albumin and complement C3 and C4 than the group of patients with history of LN and the group with SLE without renal involvement. There were no difference in kidney function and the proportion of patients with positive IgG dsDNA between active LN and history LN and SLE with no renal involvement (table 9).

Serum levels of IgE anti-dsDNA antibodies in patients with active LN, history of LN, SLE with no kidney involvement and healthy controls

A significantly higher level of IgE anti-dsDNA was detected in patients with active LN compared to controls. By contrast, there was no difference in levels of IgE anti-dsDNA between patients with a history of LN, SLE without kidney involvement and controls (table 10).

This is in line with earlier studies that report higher levels of IgE anti-dsDNA in SLE patients in general (47, 48) and in patients with LN (124). Our study extends this knowledge and includes patients with recently biopsy-verified active LN and show that IgE anti-dsDNA levels are increased in patients with active nephritis, compared to controls. By contrast, no differences in IgE anti-dsDNA were observed in patients with a history of LN, nor in patients with SLE without kidney involvement compared to controls. This observation indicates that increased levels of IgE anti-dsDNA is more linked to active kidney involvement than to the SLE disease in general. It is established that the kidney is vulnerable for hits by circulating immune complexes (198, 199).

Levels of IgE-dsDNA in correlation to C3, C4 and SLEDAI

SLE patients (n=85, 4 missing values) with C3 levels below the lower normal reference interval (<0.67 g/L) had significantly higher levels of IgE anti-dsDNA compared to SLE patients with C3 levels within the normal interval (0.67-1.43), table 11.

When patients (n=80, 9 missing values) were grouped into three groups based on the Systemic lupus erythematosus disease activity index (SLEDAI); low disease activity (SLEDAI 1-4), moderate activity (SLEDAI 5-10) and high disease activity (SLEDAI >11) there were no differences in the levels of IgE dsDNA antibodies between the groups (Figure 13). This is in contrast to previous results where a positive correlation was found between levels of IgE ds DNA and SLEDAI (200). One possible reason for discrepancy concerning SLEDAI and levels of IgE anti-dsDNA, could be the treatment regime or the proportion of patients with active LN included in the respective study populations. In the present study approximately 13 % were on current treatment with cyclophosphamide (CYC) and

seven with rituximab (rtx) at the time of sampling, compared to the study by Fujimoto in which no CYC and rtx was used (200).

Table 8 Baseline characteristics of study participants

	Active LN N= 30	**History LN N= 22**	**SLE-no nephritis N=37**	**Controls N=40**	**P-value***	**P-value****
Age, years	38 (±15)	44 (±14))	39(±14)	39 (±14)	0.487	
Women, n %	28 (93)	21 (95)	35 (94)	38 (95)		0.987
Ethnicity						
Caucasian, n (%)	30 (100)	21 (96)	35 (95)	40 (100)		0.297
Systolic BP, mmHg	123 (±21)	125 (±17)	118 (±20)	N/A	0.386	
Diastolic BP, mmHg	74 (±11)	75 (±11)	73 (±11)	N/A	0.726	
Treatment						
Prednisolone use, n (%)	28 (94)	12 (55)	27 (73)	N/A		0.005
Prednisolone dose, mg/day	10 (5-25)	3.75 (0-7.5)	5 (0-15)	N/A	0.003	
Use of antimalarial agents, n (%)	10 (33)	7 (32)	19 (51)	N/A		0.208
IS at sampling, n (%)						
Azathioprine	5 (17)	3 (14)	8 (21)	N/A		0.812
MTX	1 (3)	1 (5)	5 (13)	N/A		0.296
CYC	9 (30)	3 (14)	0 (0)	N/A		0.001
MMF	11 (37)	4 (18)	3 (8)	N/A		0.010
Rituximab	6 (20)	0 (0)	0 (0)	N/A		0.001

Data are presented as mean ± standard deviation (SD), medians (interquartile range) or numbers (percentage). BP= blood pressure, IS=immunosuppression, MTX = methotrexate, CYC= cyclophosphamide, MMF=mycophenolate mofetil

* One way ANOVA for normally distributed values and Kruskal-Wallis Test when assumption of normality is not fulfilled ** Chi-Square Test

Table 9. Comparison of laboratory and serological values

	Active LN N= 30	History LN N= 22	SLE-no nephritis N=37	P-values*	P-values**
S-creatinine (µmol/L)	64 (54-95)	67 (62-76)	65 (56-73)	0.463	
eGFR (ml/min/1.73m^2)	92.5 (63-102)	90 (75-95)	91 (80-102)	0.593	
P-albumin (g/L)	31 (28-34)	41 (37-42)	38 (33 -42)	0.000	
ESR, mm	33 (24-43)	20 (10-24)	28 (16-39)	0.011	
UACR (mg/mmol)	66 (7-125)	0.78 (0.71 -0.97)	0.6 (0.42-1.33)	0.000	
S-C3 (g/L)	0.55 (0.45 -0.76)	0.78 (0.71 -0.97)	0.72 (0.58-0.87)	0.007	
S-C4 (g/L)	0.07 (0.03-0.14)	0.14 (0.11- 0.16)	0.1 (0.07-0.14)	0.020	
IgG anti dsDNA positive, n (%)	27 (90)	20 (90.9)	37 (100)		0.150

Data are presented as medians (interquartile range) ESR= erythrocyte sedimentation rate, UACR= urine-albumin/creatinine ratio, C3= complement C3 normal range 0.67-1.43, C4= complement C4, normal range 0.12-0-32 *Kruskal-Wallis test, ** Chi-Square test

Table 10. Levels of IgE anti-dsDNA in groups of SLE patients and healthy controls

	Active LN N= 30	History LN N= 22	SLE-no nephritis N=37	Healthy controls N=40	P-values*
IgE anti-dsDNA, RU	18 (11-23)	13.5 (10-18)	14 (10-20)	11.5 (7-18)	0.029

Data are presented as medians (inter quartile range). RU= Response unit *Kruskal-Wallis Test
Post hoc analyses:
P= 0.004 Active LN vs Healthy controls
P= 0.206 Active LN vs Lupus no nephritis
P= 0.057 Active LN vs History LN
P= 0.543 History LN vs Healthy
P= 0.407 History LN vs Lupus no nephritis
P= 0.092 Healthy vs SLE no nephritis

Table 11. Levels of IgE anti-dsDNA antibodies in SLE patients grouped in low or normal C3-levels

	SLE patients (N=85)		P-values*
	C3 low (N=31)	C3 normal (N= 54)	
IgE anti-dsDNA, RU	18 (13-24)	13.5 (10-18)	0.023

Data are presented as medians (inter quartile range). RU = Response Unit
*Mann-Whitney U

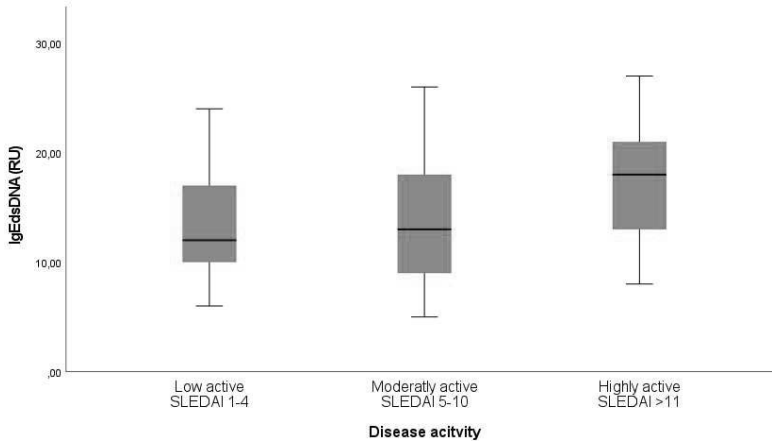


Figure 13. Levels of IgE in patients with low-, moderately- and high disease activity. No significant difference in levels of IgE anti dsDNA between patients with different disease activity, p=0.069 (KW-test). Data presented as median (middle line), interquartile range (boxes) and 95% confidence interval (whiskers). Extreme outliers (defined as Q3+3*IQ) are excluded in figure for visual presentation (p=0.160 comparing the groups without extreme outliers)

Table 12. Characteristics of the nine patients with active LN included in the experimental study of donor granulocytes exposed to sera from patients and controls

Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Age	38	58	33	31	63	25	31	35	27
Sex	F	F	M	F	F	F	F	F	F
Creatinine (µmol/L)	61	46	81	104	72	58	56	46	67
eGFR (mL/min/1.73m^2)	96	101	93	58	71	102	104	117	92
ESR, mm	71	36	24	15	19	34	16	29	-
UACR (mg/mmol)	113	6,9	33	48	3,25	0,86	7.0	7,89	109,7
dsDNA Multiplex (IE/ml)	>300	>300	>300	23	5	98	7	29	59
C3 (g/L)	0.51	0.52	0.45	0.54	1.23	0.29	0.65	0.98	0.74
C4 (g/L)	0,02	0	0,03	0,06	0,2	0,03	0,16	0,21	0,17
IgE anti-dsDNA (RU/mL)	184	33	26	13	10	13	31	21	9
SLEDAI	16	17	10	12	4	6	2	6	23
Nephritis WHO-class	III	III	III	IV/V	IV	III	V	V	V
AI/CI	9/0	3/0	8/0	4/2	10/0	5/0	1/1	0/1	0/0
Prednisone (mg)	0	0	30	20	2,5	5	10	0	15
Hydroxychloroquine	No	No	No	No	No	Yes	No	No	No
Immunosuppression*	No	No	No	Rtx	Cyc iv	AZA	MMF	Rtx	No

eGFR= estimate glomerular filtration rate, ESR=erythrocyte sedimentation rate, UACR=urine albumin/creatinine ration, RU= response units, AI=activity index, CI=chronicity index, Rtx= Rituximab, Cyc iv= cyclophosphamide intra venous pulse, AZA= Azathioprine, MMF= mycophenolate mofetil. C3=complement 3 (normal range 0.67 to 1.43 g/L). SLEDAI= systemic lupus erythematosus disease activity index, WHO= the World Health Organization *Immunosuppressive treatment at time for blood sampling

SLE sera affects donor granulocyte phenotype

Fresh granulocytes from healthy blood donor were either incubated with sera from nine patients with the active LN or with serum from controls. We selected nine patients with active LN representing a range from low to high titers of IgE anti-dsDNA for the cellular analysis. Baseline characteristic of patients are presented in table 12.

Sera from patients with active LN up-regulated CD69 to lower extent compared to sera from healthy control and down-regulate CD164 on donor basophils to higher extent compared to sera from controls (table 13). Since both CD69 and CD164 are regarded as activation markers (167, 170), sera from active LN shows a regulatory effect on basophil activation. This is in line with our data, that CD63 (representing anaphylactic degranulation) and 203c (representing piecemeal degranulation) did not show a difference between SLE sera and healthy sera (table 13).

Basophil expression of CD164 in patients with SLE has been studied previously and is postulated to enhance basophil migration to secondary lymphoid organs supporting B cell survival ant antibody production (201). To the best of our knowledge expression of CD69 on basophils in SLE has previously not been studied. However, expression of CD69 on several T-cell subsets has been described to have an immunomodulatory role in autoimmunity, and absence of CD69 may predispose to autoimmune disease (202). If basophil expression of CD69 has an immunomodulatory role in SLE remains to be elucidated.

Sera from active LN induced expression of CD15 and CD44 on donor neutrophils to a lower extent compared to sera from controls (table 13). CD15 and CD44 are both regarded as adhesion molecules (171, 203). A lower expression of CD44 on neutrophils is associated with impaired phagocytosis of apoptotic neutrophils (204). A decreased ability to clear apoptotic cells, and increased exposure of cell debris and subsequently exposure of nucleic components to the immune system, is regarded as one cornerstone in the pathophysiology of SLE (205).

CD88 on eosinophils was downregulated more by sera from controls than by sera from patients with active LN (table 13). We did not found any difference in CD15, CD44 or CD194 on eosinophils. The CD88 receptor is receptor for complement component 5a (C5a) (168). A lower expression of CD88 has been described in ANCA associated vasculitis (206). Eosinophils have not been extensively studied in pathophysiology of SLE, however a recent study demonstrated overexpression of eosinophil cationic protein in patients with SLE and (207). Further studies are needed to increase knowledge of eosinophils in patients with SLE.

Table 13. Effects on donor granulocytes after exposure to serum from patients with active LN compared to serum from controls

Granulocyte population	Active LN (n=9)	Controls (n=10)	P*
	Median (IQR)	Median (IQR)	
Basophils			
Cells upregulating CD69 (%)	4.74 (4.01-8.25)	11.12 (7.33-15.42)	**0.043**
Cells downregulating CD164 (%)	33.28 (24.54-43.69)	19.04 (13.33-30.07)	**0.035**
CD63 expression	0.97 (0.90-1.31)	1.73 (1.23-3.90)	0.065
CD203c expression	2.93 (2.51-3.45)	3.12 (3.0-4.11)	0.549
CD88 downregulation	20.86 (16.84-23.01)	15.62 (13.18-17.82)	0.315
Neutrophils			
CD44 expression (MFI)	8.11 (7.92-8.51)	9.78 (9.16-10.40)	**0.009**
CD15 expression (MFI)	138.5 (115-157)	168 (150-210)	**0.029**
CD16 expression (MFI)	11(10.20-12.80)	12.15 (10.50-12.80)	0.549
Cells downregulation CD88 (%)	77.75 (63.55-79.10)	87.97 (54.97-93.77)	0.313
Eosinophils			
Cells downregulation CD88 (%)	48.5 (43.94-52.37)	60.11 (54.32-64.25)	**0.002**
CD15 expression (MFI)	3.69 (3.32-4.43)	4.64 (3.40-4.82)	0.549
CD44 expression (MFI)	3.98 (3.79-4.03)	4.71 (4.08-7.05)	0.095
Cells downregulation CD193 (%)	11.53 (0.0-14.93)	7.88 (0.0-22.11)	0.905

To summarize, patients with active LN had higher levels of circulating IgE anti-dsDNA antibodies compared to controls. By contrast, no difference in levels of IgE anti-dsDNA was observed between patients with quiescent LN, SLE with no renal involvement and controls. In addition, SLE patients with low C3 levels had higher levels of IgE anti-dsDNA, compared to SLE patients with normal C3 levels.

Sera from active LN nephritis altered the phenotype profile of healthy donor basophils, neutrophils and eosinophils more than sera from controls, suggesting a modulatory role in disease pathogenesis.

4.2.2 Limitations

There are some limitations in our study. One limitation is the small number of patients in the cellular analysis. Another weakness is that the number of patients in the analyses of levels of IgE-dsDNA limit the possibility to detect differences between LN groups in terms of levels of IgE anti-dsDNA between patients with active LN and patients with a history of LN. All the patients were of Caucasian ancestry which reduces the generalizability of our results. It would have been of interest to know the levels of total IgE, which was not measured in this study.

4.3 STUDY III

4.3.1 Result and discussion

Study population and characteristics

At baseline, we included 49 patients with CKD stages 4-5, 54 patients with CKD stages 2-3 and 54 healthy controls. Patients with CKD had higher creatinine and lower eGFR compared to healthy controls. CRP and blood pressure was higher in patients with CKD compared to healthy controls. Bassline characteristics are shown in table 14.

After five years of follow-up, the eGFR slope was similar between CKD patients and healthy controls. CKD patients had elevated calcium and CRP compared to healthy controls, table 15.

Proteomic analyses

At baseline, we identified 19 proteins which were significantly different in patients with CKD stages 4-5 compared to in healthy controls. Proteins were grouped according to their biological, or possible pathophysiological, function: bone-metabolism, cardiovascular markers, inflammatory markers, fibrosis and kidney disease related.

During five years of follow-up we detected significant changes in 18 proteins in CKD stages 2-3 compared to healthy individuals, table 16.

Biomarkers at baseline and after five years follow-up

Based on the results from screening at baseline and after five years follow-up sCD14, ANG and OPG were chosen for further validation. Concentrations of sCD14, ANG and OPG at baseline, were significantly higher in both patient groups (i.e. CKD stages 4-5 and CKD stages 2-3) compared to healthy controls, confirming the results from proteomic analysis (figure 14a).

After five years of follow-up, a significant difference remained in the concentration of sCD14 and ANG, while OPG levels were comparable, between patients with CKD stages 2-3 and healthy individuals, figure 14b.

During the five years follow-up no significant change in sCD14, ANG and OPG were seen in patients with CKD stages 2-3 (tab 17). In healthy, ANG and OPG increased significantly.

Table **14. Baseline characteristics of study participants**

	CKD 4-5 (n=49)	**CKD 2-3 (n=54)**	**Healthy (n=54)**	**p-value ***
Age (years)	53 (40-59)	50 (38-55)	50 (39-56)	ns
Male, n (%)	29(59.2)	33 (61.1)	33 (61.1)	
eGFR (ml/min/1.73m2)	12 (9-16)	56 (50-64)	98 (86-104.5)	**<0.0001**[a]
S-Creatinine (µmol/L)	410 (334-496)	117 (106.3-129.8)	75 (67.5-81.5)	**<0.0001**[b]
S-Phosphate (mmol/L)	1.5 (1.3-1.83)	1.1 (1-1.2)	1.1 (0.99-1.2)	**<0.0001**[e]
P-hsCRP (mg/L)	1.6 (0.95-3.1)	1.8 (0.98-4)	0.86 (0.47-2.1)	**0.005**[f]
ACE-i		22(40.7)		
ARB		22 (40.7)		
Dual blockade (ACE-I + ARB)		10 (22.2)		
BMI kg/m^2	26 (23.5-28)	25 (22-28)	24 (22-27)	ns
Systolic Blood pressure (mmHg)	135 (119-149)	130 (118-140)	120 (110-129)	**<0.0001**[g] **=0.01**[h]
Diastolic Blood pressure (mmHg)	80 (75-85)	80 (70-90)	72 (65-80)	**=0.003**[i] **=0.005**[j]

Data are presented as medians (interquartile range) and number (%). eGFR=estimated glomerular filtration rate, hsCRP=high-sensitive C-reactive protein, ACE-I=angiotensin-converting enzyme inhibitor, ARB=angiotensin II receptor blocker

a: CKD 4-5 vs CKD 2-3, CKD 4-5 vs Healthy, CKD 2-3 vs Healthy, b: CKD 4-5 vs CKD 2-3, CKD 4-5 vs Healthy, CKD 2-3 vs Healthy, c: CKD 4-5 vs Healthy, d: CKD 2-3 vs Healthy

e: CKD 4-5 vs CKD 2-3, CKD 4-5 vs Healthy, f: CKD 2-3 vs Healthy, g: CKD 4-5 vs Healthy, h: CKD 2-3 vs Healthy, i: CKD 4-5 vs Healthy, j: CKD 2-3 vs Healthy

* Kruskal-Wallis test

Table 15. Laboratory values in patients and healthy controls at 5th year of follow-up

	CKD 2-3 (n=48)		Healthy (n=44)		CKD vs healthy
	At 5th year	Baseline vs 5th	At 5th year	Baseline vs 5th	
	Median (IQR)	p-value*	Median (IQR)	p-value*	p-value**
eGFR (ml/min/1.73m2)	49 (38.5-59.2)	.000	90.5 (83.2-97.7)	.004	.000
S-Creatinine (µmol/L)	129.5 (108.8-152.3)	.001	76 (69.2-83.7)	.338	.000
S-Calcium (µmol/L)	2.37 (2.32-2.41)	.001	2.31 (2.25-2.37)	.384	.002
S-Phosphate (mmol/L)	1.1 (0.94-1.2)	.938	1 (0.93-1.2)	.129	.157
P-CRP (mg/L)	1.9 (0.72-4.1)	.638	0.99 (0.53-1.95)	.468	.006

*Wilcoxon matched-paired signed test **Mann-Whitney test

Table 16. Comparing changes in protein levels from baseline to 5 years follow-up in CKD stage 2-3 and healthy

Identified proteins (gene name)	HPA ID	*p* value*
Amyloid beta precursor protein binding family A member 1 (APBA1) (APMA) (APBA1)	HPA019850	0.0003
Kruppel like factor 6 (KLF6)	HPA055831	0.002
Growth arrest specific 6(GAS6)	HPA056080	0.004
Angiogenin (ANG)	HPA036017	0.007
Solute carrier family 12 member 3 (SLC12A3)	HPA028748	0.008
Ghrelin and obestatin prepropeptide (GHRL)	HPA014246	0.008
Y-box binding protein 1 (YBX1)	HPA057159	0.009
Fibrillin 1 (FBN1)	HPA017759	0.009
Suppressor of cytokine signaling 3 (SOCS3)	HPA030890	0.015
Collagen type IV alpha 3 chain (COL4A3)	HPA042064	0.019
Cluster of differentiation 14 (CD14)	HPA002035	0.023
C-X-C motif chemokine ligand 16 (CXCL16)	HPA053253	0.026
Vascular endothelial growth factor A (VEGFA)	HPA069116	0.026
Interleukin 10 (IL-10)	HPA071391	0.027
Matrix metallopeptidase 9 (MMP9)	HPA063909	0.035
Fatty acid binding protein 1 (FABP1)	HPA028275	0.039
Collagen type IV alpha 5 chain (COL4A5)	HPA065449	0.040
Iodothyronine deiodinase 1 (DIO1)	HPA066618	0.045

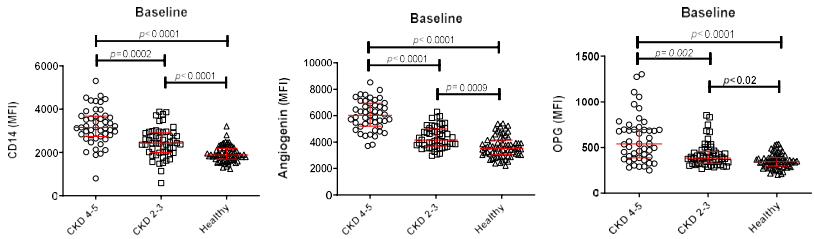
*Wilcoxon matched-paired signed rank test.

Table 17. Comparison of sCD14, ANG and OPG between baseline and 5th year of follow-up

	CKD 2–3 (*N* = 48)			Healthy (*n* = 44)		
	at baseline	t 5th year	baseline versus 5th	at baseline	at 5th year	baseline versus 5th
	median (IQR)	median (IQR)	*p* value*	median (IQR)	median (IQR)	*p* value
sCD14	2,432 (2,017–2,925)	2,472 (2,085–2,876)	0.300	1,876 (1,665–2,075)	1,799 (1,545–2,161)	0.102
ANG	4,137 (3,749–5,018)	4,302 (3,697–5,048)	0.448	3,470 (3,003–4,022)	3,282 (2,915–3,742)	**0.002**
OPG	385 (325–462)	385 (330–487)	0.156	342 (287–387)	360 (309–415)	**0.009**

*Wilcoxon matched-paired signed rank test.

A.



B

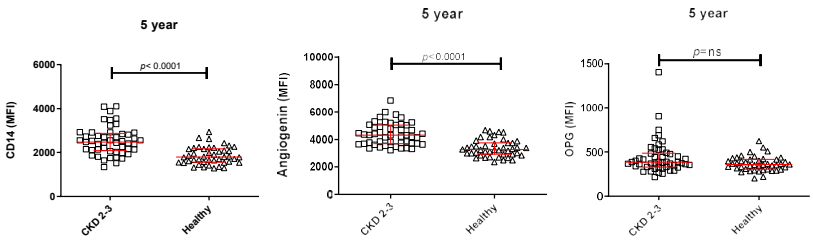


Figure 14. Levels of sCD14, angiogenin and osteoprotegerin at baseline and at 5th year of follow-up. At baseline, significantly higher levels of sCD14, angiogenin and OPG are observed in both CKD groups compared to healthy controls. A significant difference is also observed between patients with CKD stage 4-5 compared to CKD stage 2-3 (Kruskal-Wallis test, significant difference between the groups were analyzed using the post hoc multiple comparison test). B) At 5th year of follow-up, a significant difference, remains in levels of sCD14 and angiogenin comparing CKD stage 2-3 and healthy controls (Mann-Whitney U test). Scatter plots represent the 25-75 % interquartile range with whiskers and the median as the middle line.

ABI

At baseline, ABI was lower in patients with CKD stages 2-3 compared to healthy individuals. After five years of follow-up, the ABI had increased in patients with CKD stages 2-3 compared with healthy individuals who had a stable ABI during the same time. Subsequently, the difference in ABI was abolished after 5 years between the two groups (table 18).

ABI is an easy accessible and noninvasive marker of peripheral artery disease and arterial stiffness that has been suggested to be prognostic for CVD events in patients with CKD (161) . Medial arterial calcification, a known problem in patients with CKD and diabetes, can affect ABI and generate higher ABI (208, 209). In the general population, it is expected that ABI decreases by 0.025 during five years (210). ABI above the normal 1.4 is associated with increased cardiovascular mortality in patients with CKD (211). In this context, our study adds important information. During the follow-up period of five years, patients with CKD increase in ABI, remaining in the upper-normal range of ABI. Changes in ABI in the CKD group might reflect a calcification process of the arterial media layer. The aspect of media arterial calcification needs to be taken into consideration when interpreting upper-normal ABI values in earlier stages of CKD.

Table 18 Ankle-brachial index (ABI) and carotid media intima thickness (CIMT) in patients with CKD stage 2-3 and healthy individuals

	CKD 2-3 (N=48)			**Healthy (n=44)**			**CKD vs Healthy**	
	At baseline	At 5th year	Baseline vs 5th	At baseline	At 5th year	Baseline vs 5th	At baseline	At 5th year
	Median (IQR)	Median (IQR)	*p-value*	Median (IQR)	Median (IQR)	*p-value*	*p-value*	*p-value*
ABI	1.08 (1.03-1.13)	1.16 (1.10-1.24)	**.001***	1.16 (1.09-1.23)	1.15 (1.1-1.2)	ns	**.0002****	ns
CIMT (mm^2)	0.63 (0.57-0.69)	0.63 (0.58-0.70)	ns	0.64 (0.54-0.75)	0.69 (0.61-0.79)	**.0005***	ns	**.03****

*Wilcoxon matched-paired signed test **Mann-Whitney test

ABI in correlation to sCD14 and OPG

sCD14 and OPG were positively correlated with ABI at five years follow-up in patients with CKD 2-3 but not in healthy subjects (Figure 15). These findings are in line with previous studies which reported a positive correlation between OPG and sCD14 and arterial stiffness measured as pulse wave velocity (212, 213).

Additionally, we showed that changes in sCD14 and changes in ABI over a period of five years were significantly correlated (Figure 16).

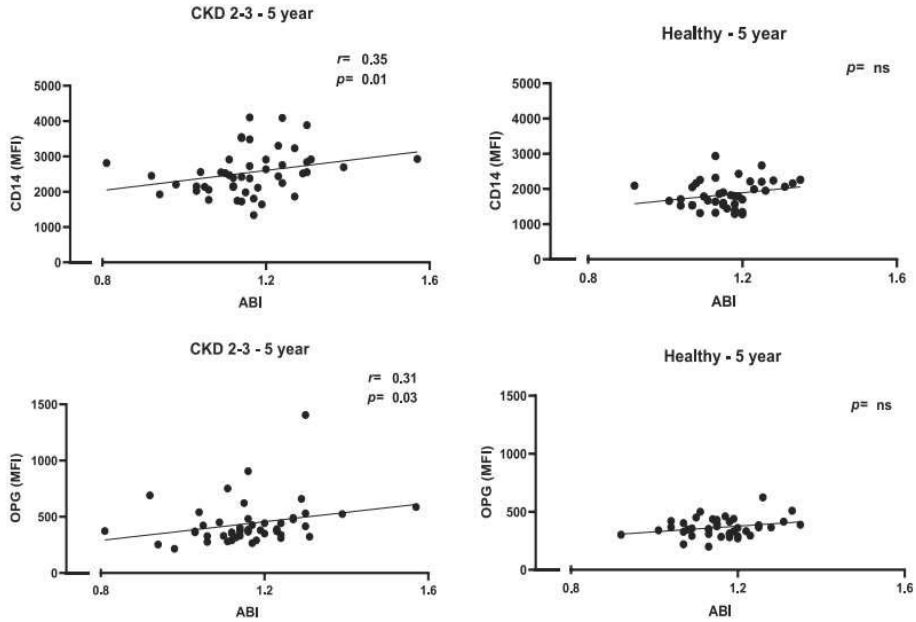


Figure 15 Correlations between sCD14 levels, OPG levels and ABI at 5th year of follow-up. Calculated using the Spearman´s rank correlation test.

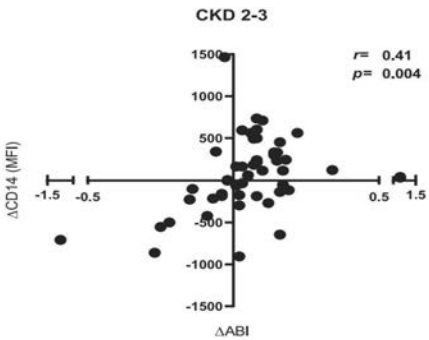


Figure 16. Correlation of changes in sCD14 and ABI during 5 years. Correlation between changes in sCD14 levels (ΔCD14) and changes in ABI (ΔABI) over period of 5 years in patients with CKD 2-3 using the Spearman´s rank correlation test.

OPG in relation to CIMT

Results of CIMT in the present patient cohort have been published earlier (214). CIMT increased in healthy individuals during the five years of follow-up, but not in patients with CKD (p=0.0005 and p=ns respectively), and was significantly higher in healthy subjects after five years (p=0.03) table 18.

In present study, we extend these findings and investigate the correlation between CIMT and OPG. Increasing levels of OPG correlated with increasing CIMT in both healthy subjects and patients with CKD stages 2-3 at baseline (Figure 17). A correlation between OPG and CIMT has previously been reported in the general population as well as in patients with coronary artery disease (215, 216).

During the five years of follow-up, the correlation between OPG and CIMT was abolished in our study. This may reflect the interference of age in the association between OPG and CIMT, as implicated by previous studies showing that an increase OPG in a younger population does not support an ongoing atherosclerosis (216). Another possible interpretation is a dual biological role of OPG. OPG has been attributed a protective role by supporting survival of endothelial- and vascular smooth muscle cells (216). Also, several mouse studies implicate a protective role in vascular calcification (217).

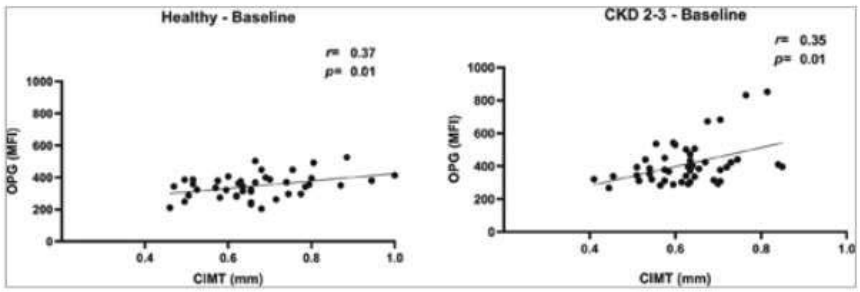


Figure 17. Correlation between OPG and CIMT in patients with CKD stages 2-3 and healthy at baseline. With Spearman´s rank correlation test.

To summarize, in comparison with healthy individuals, patients with CKD stages 2-3 had increased levels of sCD14, OPG and ANG at baseline. After five years of follow-up levels of sCD14 and ANG were still higher in patients with CKD stages 2-3. In patients with CKD stages 2-3, increased levels of sCD14 and OPG were associated with ABI at the 5th year of follow-up.

4.3.2 Limitations

There are some methodological considerations. First, ABI can give falsely high values in patients with media calcification. Patients with diabetes and CKD are known to be at risk of media calcification as discussed above. In patients on hemodialysis treatment, toe-brachial-index or exercise-ABI has been suggested as a better alternative in comparison to ABI (218). Possibly toe-brachial-index is also more accurate in earlier stages of CKD (218), and could have given additional prognostic information of vascular lesions. Second, we investigated atherosclerosis in carotid artery by assessing carotid intima-media thickness (CIMT). Arteriosclerosis, as a measurement of arterial stiffness and vascular calcification, can be assessed with pulse wave velocity (PWV) and is reported to have advantages in assessing the cardio vascular risk (219). At the time of inclusion, we were not able to perform PWV

Thirdly, the proteomic procedure is time consuming and renders semi quantitative values of protein levels. Changes in protein levels needs to be verified with a method that offers quantitative analysis. Since many proteins are included there is a risk of significance due to multiple testing which was addressed by correcting for multiple testing with Bonferroni correction.

4.4 Study IV

4.4.1 Result and discussion

Study population and baseline characteristics

We included 110 patients with COVID-19 infection admitted to Danderyd University Hospital, Stockholm, Sweden. The majority of patients (n=95) were hospitalized at a general ward, 11 patients were at an intermediate care unit and 4 patients at an intensive care unit. We also included 33 sex- and eGFR (but not age) matched patients with mild-to-severe CKD without COVID-19 infection. In addition, we included 35 healthy controls, without COVID-19 infection, which were sex- and age matched with COVID-19 patients. Patients with COVID-19 were older and had a higher BMI compared to the two control groups. eGFR was higher in the COVID-19 group compared to CKD-controls, but lower than in healthy controls. CRP was higher in COVID-19 compared to both control groups. MIP-1α was lower, but IL-6 and sCD14 were higher in COVID-19 compared to both control groups. No difference in levels of MCP-1 was observed. Comparisons between study participants are presented in table 19.

Next, we compared the values of MCP-1, MIP-1α, IL-6 and sCD14 in patients with COVID-19 infection (n=33) with patients with CKD without infection (table 20). Groups were matched for eGFR and sex (but not age). A significant difference remained between concentrations of MIP-1α, IL-6 and sCD14. This suggests that differences in levels of monocyte modulators is due to COVID-19 and not a consequence of a decline in kidney function.

Table 19. Comparisons between study groups

	COVID-19 (N = 110)		CKD patients (N = 33)		Healthy subjects (N = 35)		
	Median	IQR	Median	IQR	Median	IQR	P^*, *K-W*
Age (y)	60	50-69	55	45-58	50	39-57	<.001[a]
BMI (kg/m^2)	28	25-32	25	24-28	24	22-27	<.001[b]
Creatinine (μmol/L)	73	58-89	122	107-160	72	66-77	<.001[c]
eGFR (mL/min/1.73 m^2)	84	67-90	53	40-65	102	96-108	<.001[d]
Potassium (mmol/L)	3.9	3.6-4.2	4.3	4.0-4.4	4.0	3.9-4.2	<.001[e]
CRP (mg/L)	99	63-174	1.6	0.9-4.3	0.89	0.31-2.2	<.001[f]
Monocyte recruitment (chemoattractants)							
MCP-1 (pg/mL)	350	234-512	362	264-462	306	230-425	NS
MIP-1α (pg/mL)	320	269-379	385	350-473	385	323-577	<.001[g]
IL-6 (pg/mL)	27.8	13.2-58.9	4.7	2.5-14.4	2.5	1.7-10.2	<.001[h]
Monocyte function (immune modulating mediator)							
sCD14 (ng/mL)	2094	1557-2559	1098	935-1260	853	750-923	.001[i]

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein; sCD14, soluble cluster of differentiation 14.

*Kruskal-Wallis test.

Post hoc analyses:

[a] $P < .01$ comparing COVID-19 and CKD patients; $P < .0001$ comparing COVID-19 and healthy controls.

[b] $P < .05$ comparing COVID-19 and CKD patients; $P < .0001$ comparing COVID-19 and healthy controls.

[c] $P < .0001$ comparing COVID-19 and CKD patients; NS comparing COVID-19 and healthy controls.

[d] $P < .0001$ comparing COVID-19 and CKD patients; $P < .0001$ comparing COVID-19 and healthy controls,

[e] $P < .0001$ comparing COVID-19 and CKD patients; $P < .05$ comparing COVID-19 and healthy controls.

[f] $P < .0001$ comparing COVID-19 and CKD patients; $P < .001$ comparing COVID-19 and healthy controls.

[g] $P < .0001$ comparing COVID-19 and CKD patients; $P < .001$ comparing COVID-19 and healthy controls.

[h] $P < .0001$ comparing COVID-19 and CKD patients; $P < .001$ comparing COVID-19 and healthy controls.

[i] $P < .0001$ comparing COVID-19 and CKD patients; $P < .001$ comparing COVID-19 and healthy controls.

Table 20. Subgroup of patients with COVID-19 eGFR matched with CKD patients without COVID-19 infection

	COVID-19 (N = 33)		CKD (N = 33)		
	Median	IQR	Median	IQR	P[a]
Age (y)	68.0	55.5-80.5	55.0	44.5-58.0	<.001
BMI (kg/m^2)	27.5	25.7-30.0	25.0	23.5-28.0	.05
Creatinine (µmol/L)	1.10	0.92-1.55	1.38	1.21-1.81	.01
eGFR (mL/min/1.73 m^2)	55.0	39.5-68.0	53.0	40-65	NS
Potassium (mmol/L)	4.0	3.8-4.2	4.3	4.0-4.4	<.05
CRP (mg/L)	98.0	67.5-200.8	1.6	0.9-4.3	<.001
Monocyte recruitment (chemoattractant)					
MCP-1 (pg/mL)	405	235-574	362	264-462	NS
MIP-1α (pg/mL)	332	285-409	385	349.6-472.6	<.05
IL-6 (pg/mL)	36.7	14.5-63.8	4.7	2.5-14.4	<.001
Monocyte function (immune modulating mediator)					
sCD14 (ng/mL)	2394	2018-2762	1098	935-1260	<.001

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein; sCD14, soluble cluster of differentiation 14.
[a]Mann-Whitney *U* test.

In-hospital mortality in COVID-19 patients

Patients with COVID-19 infection (n=115), who died in hospital (n=15) were predominantly male and older compared to patients who survived. There were also differences in laboratory values between deceased and surviving patients, i.e. patients who died had lower eGFR, higher CRP, WBC count, MIP-1α and IL-6, table 21.

Unadjusted and adjusted risk of in-hospital mortality with COVID-19

We used multiple logistic regression to analyze in-hospital mortality. In an unadjusted model age, eGFR, CRP, WBC count, MCP-1, MIP-1α and IL-6 were associated with in-hospital mortality in patients with COVID-19, table 22. The regression model was then adjusted for age, sex and eGFR. In the adjusted model CRP, WBC count, MCP-1 and MIP-1α remained associated with an increased risk of in-hospital mortality table 22. The adjusted model needs to be interpreted with caution since there was a small number of events.

Kaplan-Meier plot (figure 18) for cumulative survival adjusted for age shows a relationship between MIP-1α levels above or under median, in relation to survival (p=0.042).

Risk of COVID-19 in-hospital mortality for patients with lower eGFR

Next we were interested to explore mortality risk in COVID-19 in relation to eGFR. Based on the median eGFR (84 ml/min/1.73m^2) in the study population, we analyzed mortality in patients with eGFR below 84 ml/min/1.73m^2 in an unadjusted logistic regression. Age, higher WBC count, and higher level of MIP-1α were significantly associated with in-hospital mortality, table 23.

To summarize, we found that the levels of MCP-1 and MIP-1α in peripheral blood were associated with in-hospital mortality in patients with COVID-19. Our results demonstrated, apart from kidney function, MCP-1 and MIP-1α levels provided additional prognostic information to previously recognized risk factors. In addition, results in present study highlight the impact of monocyte recruitment mediators to the development of COVID-19-associated complications.

Table 21. Characteristic (median values) of patients who died (n=15) and survived (n=95) in hospital

	Survived (%)	Deceased (%)	P^a
Gender			
Female	41	13	<.05
Male	59	87	
CKD stage			
0	47	20	<.01[b]
2	40	33	
3	6	40	
4	4	7	
5	2	0	
Age (y), median (IQR)	58.0 (48.0-67.0)	69 (61-82)	.01
BMI (kg/m^2), median (IQR)	28.4 (24.5-31.8)	26.5 (25.5-27.5)	.01
Creatinine (mg/dL), median (IQR)	70.5 (55.0-83.0)	92.0 (77.0-111.0)	<.01
eGFR ($mL/min/1.72\,m^2$), median (IQR)	87.0 (71.0-83.0)	62.0 (45.0-75.0)	<.05
CRP (mg/L), median (IQR)	93.5 (60.0-155.5)	180.5 (106.8-298.5)	<.05
WBC count ($\times 10^9$/L), median (IQR)	6.1 (4.6-8.0)	10.1 (7.5-14.3)	<.01
MCP-1 (pg/mL)	330 (228-457)	560 (307-938)	NS
MIP-1α (pg/mL)	309 (267-371)	389 (320-436)	<.05
IL-6 (pg/mL)	23.4 (13.0-55.0)	58.8 (47.1-126.2)	<.05
sCD14 (ng/mL)	2075 (1523-2497)	2124 (1819-2186)	NS

Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; IQR, interquartile range; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein; sCD14, soluble cluster of differentiation 14; WBC, white blood cell.

[a]Chi-square analysis.

[b]Fisher's exact test.

Table 22 Risk of in-hospital mortality in patients with COVID-19

	Unadjusted analysis			Adjusted for age, sex and eGFR		
	P	OR	95% CI	*P*	OR	95% CI
Age (y)	<.01	1.071	1.023-1.122			
Sex (male)	.055	4.527	0.967-21.196			
BMI (kg/m^2)	NS	0.960	0.867-1.064	NS	0.978	0.864-1.107
CRP (mg/L)	<.01	1.009	1.003-1.015	<.05	1.007	1.001-1.014
WBC count ($\times 10^9$/L)	<.001	1.292	1.116-1.497	.001	1.462	1.164-1.836
eGFR ($mL/min/1.73\,m^2$)	<.01	0.969	0.947-0.991			
Monocyte recruitment (chemoattractants)						
MCP-1 (pg/mL)	<.05	1.001	1.000-1.002	<.05	1.001	1.000-1.002
MIP-1α (pg/mL)	<.05	1.005	1.001-1.010	<.01	1.007	1.002-1.012
IL-6 (pg/mL)	<.05	1.006	1.000-1.012	NS	1.006	0.999-1.012
Monocyte function (immune modulating mediator)						
sCD14 (ng/mL)	NS	1.000	1.000-1.000	NS	1.000	1.000-1.000

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein; sCD14, soluble cluster of differentiation 14; WBC, white blood cell.

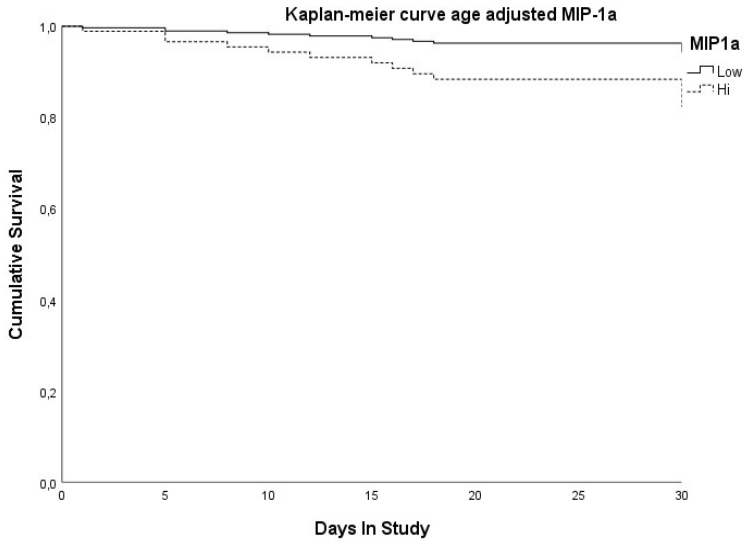


Figure 18. 30 days survival plot, adjusted for age, for proportion of survival of patients with MIP-1 α above or under the median MIP-1 α.

Table 23. Risk of in-hospital mortality in COVID-19 in patients with eGFR below 84 ml/min/1.73m^2

	OR	**P-value***
	(95% C.I.)	
Age	1.079 (1.008-1.155)	0.028
WBC count	1.206 (1.034-1.408)	0.017
MIP-1α	1.010(1.000-1.019)	0.043

WBC= white blood cell, MIP-1α=monocyte OR= odds ratio, CI=confidence interval,

*multiple logistic regression

4.4.2 Limitations

There are limitations in this study. One limitation is the small patient sample size. The adjusted models need to be interpreted with caution since there was a small number of events and a limited patient sample size. There is a small risk for undiagnosed bacterial infection which might affect interpretation of the results. Also, we did not have information about kidney function in patients before COVID-19 infection and urine samples were not obtained. Therefore, we are not able to distinguish if the increased creatinine levels are due to COVID-19 and acute kidney injury or presence of CKD.

5 CONCLUSIONS

- Patients with IgAN have an altered immunological phenotype of B cell and composition of monocyte subsets. Based on our results, changes in B cell phenotype are not maintained by cytokines affecting B cell survival, rather by changes in balance between B and T cell subsets implying the importance of T cell dependent B cell maturation. Determining B cell subsets might be of importance in future evaluation of B cell targeted therapies in IgAN, especially in terms of number of long-lived B cells, which cannot be targeted through anti-CD20 treatment.

- SLE patients with active, but not quiescent LN or SLE with no kidney involvement, have elevated levels of IgE anti-dsDNA antibodies. Comparing SLE patients with low or normal C3 levels, we report higher levels of IgE anti-dsDNA in patients with low C3 levels, but no difference in IgE ant-dsDNA levels between patients with different global disease activity assessed by SLEDAI-score. This opens for future studies evaluating if IgE mediated mechanisms are prone to increase the total burden of circulating immune complexes in SLE with active LN and if IgE-dsDNA can be connected to tissue damage.
  We show that sera from active LN nephritis alters the phenotype profile of healthy donor basophils, neutrophils and eosinophils, suggesting a modulatory role in disease pathogenesis.

- Increased levels of sCD14 and OPG were associated with arterial stiffness evaluated by ABI in patients with mild-to-moderate CKD, implying their role as possible biomarkers of early vascular lesions in CKD. Further studies are needed to understand the potential mechanism of sCD14 and OPG in disease pathogenesis, or if they can contribute as early detection markers in diagnosis of vascular disease.

- Monocyte chemoattractants MCP-1 and MIP-1α provide addditional prognostic information in hospitalized patients with COVID-19, and this is valid for both patients with normal and impaired kidney function.

6 POINTS OF PERSPECTIVE

We have demonstrated a lower ratio between plasmablasts/Th2 and pre-switched B cells/Th2 in IgAN compared to healthy. This indicates a changed balance between B cell subsets and Th2 in patients with IgAN. Th2 cytokines (e.g. IL-4, IL-13) mediate the humoral response and glycosylation of IgA (182, 183). How the immunological crosstalk that favors a Th2 skewed immune response in IgAN operates, is not fully delineated. The potential role of basophils in the pathophysiology of IgAN have not yet been studied. It would be of interest to extend our findings from study II to explore a potential involvement of basophils in the pathophysiology of IgAN. The same experimental protocol can be used, with patient serum and healthy donor basophils.

Production of GdIgA, along with production of autoantibodies targeted against GdIgA, are central in the pathophysiology of IgAN. Antibodies, both auto-antibodies and part of the normal humoral response, are produced by both plasmablasts and short- and long-lived plasma cells (220). The source of GdIgA in IgAN is still unknown. CD19- long-lived cells constitute a part of the long-lived plasma cell pool, and as such, contributes to antibody production (221). Based on the results in present thesis of a higher proportions of CD19- long-lived cells in IgAN it is of interest to investigate if levels of GdIgA correlate with proportions of CD19 -long lived plasma cells and disease progression.

In current thesis we show SLE sera can affect the granulocyte phenotype. To further understand if IgE anti-dsDNA in serum can contribute to changes in granulocyte phenotype, it would be of interest to repeat our cellular experiments and include SLE sera depleted of IgE. In addition, we would like to extend our knowledge on how basophil phenotype is affected after transmigration into various tissues in SLE. It is known that basophils become activated when they transmigrate through the endothelium, a process that can be achieved ex-vivo by cross-linking of their adhesion molecules CD62L, CD11b and CD49d (222). In addition, kidney biopsies can be double stained for immune complexes and basophil infiltration to identify if their respective location in kidney biopsies overlap.

7 ACKNOWLEDGEMENTS

This thesis would not have been possible without all the incredible people around me and I am deeply thankful for all your support. My time as PhD student can in a way be resembled with a marathon: there were peaks and valleys, sometimes the tour demanded more intensive sprints and sometime the path forward turned out to be a blind alley. Yet, on occasions the runner's high appeared, and then everything felt possible.

My most sincere and special gratitude to;

Joachim Lundahl, my main supervisor, for showing the way forward and setting the pace, with a perfect balance between sprints and jog, and never-ending smart ideas and a smile. For learning me about research, immunology and scientific networking. Thank you for always making time for my questions, alongside of everything you do. I still don´t understand how you manage it all, it's amazing what one can achieve on a 32 hour day.

Stefan Jacobson, co-supervisor, for constantly having our patients' best at front and reminding what the marathon is all about, you truly are a great role model as nephrologist, scientist and leader. Many a time during this race your words from the very first research meeting, have echoed in my head: over and above, remember this is about mastering endurance. Thank you for being supportive, helping, available and optimistic and for introducing me for some of your research collaborators.

Anna Nopp, co-supervisor, for knowing all the marathon rules at KI and for welcoming me to the research group. Thank you for our early mornings in the former lab, for providing the structure in our research group, for your wise thoughts, sharp eyes and kind support along the run.

Thank you:

Present and former members of the research group at KI/KI-SÖS; **Ladan Mansouri**, postdoc and dear friend, for all the support, great collaboration and for learning me about laboratory work. Without you this run would not have been as fun. I admire your strength, intelligence and kind heart. **Caroline Nilsson**, **Ulrika Hellberg**, **Carina Uhl**, **Helena Thulin**, **Frida Kalm** and **Zenib Aljadi** for great feed-back and help along the way and for interesting scientific discussions. Thank you **Zekiye** for splendid laboratory work and for always being flexible and ready to handle all the samples.

Sigrid Lundberg, mentor and co-author, for your support and valuable advices during this time and for all your positive energy. I really enjoyed our IIgANN conference in Argentina and networking with IgAN colleagues from around the world.

To my co-authors **Iva Gunnarsson**, **Elisabet Svenungsson**, **Britta Hylander**, **Jochen M. Schwenk**, **Mun-Gwan Hong**, **Maria J. Eriksson**, **Sebastian Havervall** and **Charlotte Thålin** for all your hard work, great generosity and collaboration.

To **Tomas Jernberg**, **Håkan Wallén**, **Nina Ringart**, **Malin Wirf**, **Siw Svensson** , the Karolinska Institutet, Department of Clinical Sciences, Danderyd Hospital (KIDS), for the structure during my time as PhD-student. **Fredrik K. Johansson**, medical statistician also KIDS, thank you for assistance during the work with Study IV.

To all my hardworking, helpful, supporting and smart colleagues in The Department of Nephrology at Danderyd University Hospital. I learn from you every day and I truly appreciate to share big and small

clinical moments with each and every one of you: **Eva, Boa, Fredrik, Marie, Anna, Bodil, Lars, Niklas, Barbara Julia, Shervin, Haben, Rebecca, Saba, Shayan, Sören** and **Jens**. And to former colleagues **Lysann, Tora, Josefin, Bodil, Frida, Guna and Anders.** I would like to express an extra gratitude to **Björn Samnegård**, and **Annika Alvelius**, for giving me the opportunity to combine clinical work with research. **Elena Fedchenko**, my clinical supervisor, thank you for supporting me, for always taking time to answer all my questions. I admire your knowledge and great care of your patients. **Christina Montgomerie**, thank you for making an extra effort to provide research time when I needed it the most. **Karin Bergen**, I really appreciate all the advices you gave during my last period as a PhD-student, and thank you so much for last minute language editing. **Mazdak Nurmi**, thank you for walking the extra mile, in search for research samples.

To all the nurses at The Department of Nephrology for helping me during sample collection Especially **Jeanette Wallin**, former research nurse, I am so grateful for everything you helped me with during study I. It was great to be roommates, I learned a lot from you.

To **Anna Sjöström**, PhD-student and research school mate, for interesting and inspiring time during and after KI´s research school. You have achieved so much during your time as a PhD-student, you bright research star!

Mr&Mrs Halonen, thank you for in-put representing the readers of Popular Science Summary.

My dear friends from Medical School, Uppsala University; **Caroline**, **Shetha**, **Sarah**, **Sara**, **Anna-Karin** and **Pernilla**. So many good memories and fun times! I always look forward to spend time with you.

Parisa and **Camilla**, you give me energy in so many ways! I long for more tours to the forest and relaxing afternoon tea times.

Therese and **Camilla**, it is always nice when we get together! I am excited about the postponed weekend trip.

Jessica, my running mate and Skalman, I look forward future adventures tours. You are such an inspiring good-life connoisseur.

Kattis, my dear, dear friend. So many great memories, and so many more to come. You have always been there and for that, I am so grateful!

Anders, for your love and support and for being a solid rock to lean on. For all the extra work that you had to handle with our children and household during the last time. I promise to be more enthusiastic about various constructions and not to blunder your calendar with “dagis har stängt, din tur igen”.

Isak, Erwin och **Selma**, älskade och underbara barn! Tack för den glädje ni sprider och för att ni ger mig perspektiv. Ni är det som jag är överlägset mest stolt och tacksam för i hela världen <3

Mama i **tata**, od srca vam hvala za vasu ljubav i beskrajnju podrsku! Hvala vam sto ste se uvijek, kao i sada takodjer, borili za mene i za nas. Veoma sam sretna sto vas imam

I am grateful to all the patients and healthy volunteers for your effort and generosity to participate. These studies could not have been done without you!

This work was supported by founding from Stockholm County Council ALF, The Swedish Renal Foundation Njurfonden, Swedish Society of Nephrology, Swedish Society of Medicine, The Hesselman´s Research Foundation, Knut and Alice Wallenberg Foundation, the SciLifeLab and the Jonas and Christina af Jochnick Foundation.

8 REFERENCES

1. Romagnani P, Remuzzi G, Glassock R, Levin A, Jager KJ, Tonelli M, et al. Chronic kidney disease. Nature reviews Disease primers. 2017;3:17088.

2. KDIGO. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group

KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease

Kidney Int Suppl, 3 (1) (2013), pp. 1-150.

3. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011;80(1):17-28.

4. Summary of Recommendation Statements. Kidney Int Suppl (2011). 2013;3(1):5-14.

5. Björk J, Grubb A, Larsson A, Hansson LO, Flodin M, Sterner G, et al. Accuracy of GFR estimating equations combining standardized cystatin C and creatinine assays: a cross-sectional study in Sweden. Clin Chem Lab Med. 2015;53(3):403-14.

6. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211-59.

7. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet. 2017;389(10075):1238-52.

8. SNR. Svensk Njurregister Årsrapport 2020.

9. Rodrigues JC, Haas M, Reich HN. IgA Nephropathy. Clin J Am Soc Nephrol. 2017;12(4):677-86.

10. Floege J, Amann K. Primary glomerulonephritides. Lancet. 2016;387(10032):2036-48.

11. Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. Geneva, World Health Organization; 2020. A summary of data sources and methods is available.

12. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351(13):1296-305.

13. Staunstrup NH, Petersen CC, Fuglsang T, Starnawska A, Chernomorchenko A, Qvist P, et al. Comparison of electrostatic and mechanical cell sorting with limited starting material. Cytometry Part A : the journal of the International Society for Analytical Cytology. 2022;101(4):298-310.

14. Saritas T, Floege J. Cardiovascular disease in patients with chronic kidney disease. Herz. 2020;45(2):122-8.

15. Thompson S, James M, Wiebe N, Hemmelgarn B, Manns B, Klarenbach S, et al. Cause of Death in Patients with Reduced Kidney Function. J Am Soc Nephrol. 2015;26(10):2504-11.

16. Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimbürger O, Massy Z. Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol. 2008;3(2):505-21.

17. Naqvi SB, Collins AJ. Infectious complications in chronic kidney disease. Adv Chronic Kidney Dis. 2006;13(3):199-204.

18. Oetjens MT, Luo JZ, Chang A, Leader JB, Hartzel DN, Moore BS, et al. Electronic health record analysis identifies kidney disease as the leading risk factor for hospitalization in confirmed COVID-19 patients. PLoS One. 2020;15(11):e0242182.

19. Gansevoort RT, Hilbrands LB. CKD is a key risk factor for COVID-19 mortality. Nature reviews Nephrology. 2020;16(12):705-6.

20. Flythe JE, Assimon MM, Tugman MJ, Chang EH, Gupta S, Shah J, et al. Characteristics and Outcomes of Individuals With Pre-existing Kidney Disease and COVID-19 Admitted to Intensive Care Units in the United States. Am J Kidney Dis. 2021;77(2):190-203.e1.

21. Vaziri ND, Pahl MV, Crum A, Norris K. Effect of uremia on structure and function of immune system. J Ren Nutr. 2012;22(1):149-56.

22. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, et al. Aspects of immune dysfunction in end-stage renal disease. Clin J Am Soc Nephrol. 2008;3(5):1526-33.

23. Karadsheh FF, Weir MR. Chapter 23 - Hematologic Complications of Chronic Kidney Disease: Leukocyte and Monocyte Function. In: Kimmel PL, Rosenberg ME, editors. Chronic Renal Disease. San Diego: Academic Press; 2015. p. 277-84.

24. Hiepe F, Radbruch A. Plasma cells as an innovative target in autoimmune disease with renal manifestations. Nature reviews Nephrology. 2016;12(4):232-40.

25. Pakkanen SH, Kantele JM, Moldoveanu Z, Hedges S, Hakkinen M, Mestecky J, et al. Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. Clin Vaccine Immunol. 2010;17(3):393-401.

26. Rickert RC. New insights into pre-BCR and BCR signalling with relevance to B cell malignancies. Nat Rev Immunol. 2013;13(8):578-91.

27. Espi M, Koppe L, Fouque D, Thaunat O. Chronic Kidney Disease-Associated Immune Dysfunctions: Impact of Protein-Bound Uremic Retention Solutes on Immune Cells. Toxins (Basel). 2020;12(5).

28. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. Nature reviews Nephrology. 2013;9(5):255-65.

29. Kong BS, Kim Y, Kim GY, Hyun JW, Kim SH, Jeong A, et al. Increased frequency of IL-6-producing non-classical monocytes in neuromyelitis optica spectrum disorder. J Neuroinflammation. 2017;14(1):191.

30. Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. Front Immunol. 2013;4:23.

31. Hijdra D, Vorselaars AD, Grutters JC, Claessen AM, Rijkers GT. Phenotypic characterization of human intermediate monocytes. Front Immunol. 2013;4:339.

32. Ożańska A, Szymczak D, Rybka J. Pattern of human monocyte subpopulations in health and disease. Scand J Immunol. 2020;92(1):e12883.

33. Naicker SD, Cormican S, Griffin TP, Maretto S, Martin WP, Ferguson JP, et al. Chronic Kidney Disease Severity Is Associated With Selective Expansion of a Distinctive Intermediate Monocyte Subpopulation. Front Immunol. 2018;9:2845.

34. Heine GH, Ulrich C, Seibert E, Seiler S, Marell J, Reichart B, et al. CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. Kidney Int. 2008;73(5):622-9.

35. Ulrich C, Heine GH, Seibert E, Fliser D, Girndt M. Circulating monocyte subpopulations with high expression of angiotensin-converting enzyme predict mortality in patients with end-stage renal disease. Nephrol Dial Transplant. 2010;25(7):2265-72.

36. Al-Chaqmaqchi HA, Moshfegh A, Dadfar E, Paulsson J, Hassan M, Jacobson SH, et al. Activation of Wnt/β-catenin pathway in monocytes derived from chronic kidney disease patients. PLoS One. 2013;8(7):e68937.

37. Wallquist C, Mansouri L, Norrbäck M, Hylander B, Jacobson SH, Lundahl J. Early Changes in Monocyte Adhesion Molecule Expression and Tumor Necrosis Factor-α Levels in Chronic Kidney Disease - A 5-Year Prospective Study. Am J Nephrol. 2016;44(4):268-75.

38. Verkade MA, van Druningen CJ, Vaessen LM, Hesselink DA, Weimar W, Betjes MG. Functional impairment of monocyte-derived dendritic cells in patients with severe chronic kidney disease. Nephrol Dial Transplant. 2007;22(1):128-38.

39. Wallquist C, Paulson JM, Hylander B, Lundahl J, Jacobson SH. Increased accumulation of CD16+ monocytes at local sites of inflammation in patients with chronic kidney disease. Scand J Immunol. 2013;78(6):538-44.

40. Lim WH, Kireta S, Leedham E, Russ GR, Coates PT. Uremia impairs monocyte and monocyte-derived dendritic cell function in hemodialysis patients. Kidney Int. 2007;72(9):1138-48.

41. Bowe B, Xie Y, Xian H, Li T, Al-Aly Z. Association between Monocyte Count and Risk of Incident CKD and Progression to ESRD. Clin J Am Soc Nephrol. 2017;12(4):603-13.

42. Cox SN, Serino G, Sallustio F, Blasi A, Rossini M, Pesce F, et al. Altered monocyte expression and expansion of non-classical monocyte subset in IgA nephropathy patients. Nephrol Dial Transplant. 2015;30(7):1122-232.

43. Esteve Cols C, Graterol Torres FA, Quirant Sanchez B, Marco Rusinol H, Navarro Diaz MI, Ara Del Rey J, et al. Immunological Pattern in IgA Nephropathy. Int J Mol Sci. 2020;21(4).

44. Ducrest S, Meier F, Tschopp C, Pavlovic R, Dahinden CA. Flowcytometric analysis of basophil counts in human blood and inaccuracy of hematology analyzers. Allergy. 2005;60(11):1446-50.

45. Falcone FH, Knol EF, Gibbs BF. The role of basophils in the pathogenesis of allergic disease. Clin Exp Allergy. 2011;41(7):939-47.

46. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. J Allergy Clin Immunol. 2018;142(2):370-80.

47. Charles N, Hardwick D, Daugas E, Illei GG, Rivera J. Basophils and the T helper 2 environment can promote the development of lupus nephritis. Nat Med. 2010;16(6):701-7.

48. Atta AM, Sousa CP, Carvalho EM, Sousa-Atta ML. Immunoglobulin E and systemic lupus erythematosus. Braz J Med Biol Res. 2004;37(10):1497-501.

49. Pellefigues C, Charles N. The deleterious role of basophils in systemic lupus erythematosus. Curr Opin Immunol. 2013;25(6):704-11.

50. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13(3):159-75.

51. Chonchol M. Neutrophil dysfunction and infection risk in end-stage renal disease. Seminars in dialysis. 2006;19(4):291-6.

52. Pindjakova J, Griffin MD. Defective neutrophil rolling and transmigration in acute uremia. Kidney Int. 2011;80(5):447-50.

53. Sardenberg C, Suassuna P, Andreoli MC, Watanabe R, Dalboni MA, Manfredi SR, et al. Effects of uraemia and dialysis modality on polymorphonuclear cell apoptosis and function. Nephrol Dial Transplant. 2006;21(1):160-5.

54. Moshfegh A, Jacobson SH, Halldén G, Thylén P, Lundahl J. Impact of hemodialysis membrane and permeability on neutrophil transmigration in vitro. Nephron. 2002;91(4):659-65.

55. Frangou E, Vassilopoulos D, Boletis J, Boumpas DT. An emerging role of neutrophils and NETosis in chronic inflammation and fibrosis in systemic lupus erythematosus (SLE) and ANCA-associated vasculitides (AAV): Implications for the pathogenesis and treatment. Autoimmunity reviews. 2019;18(8):751-60.

56. Mahajan A, Herrmann M, Muñoz LE. Clearance Deficiency and Cell Death Pathways: A Model for the Pathogenesis of SLE. Front Immunol. 2016;7:35.

57. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. J Immunol. 2010;184(6):3284-97.

58. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol. 2011;187(1):538-52.

59. Nakayama F, Nishihara S, Iwasaki H, Kudo T, Okubo R, Kaneko M, et al. CD15 expression in mature granulocytes is determined by alpha 1,3-fucosyltransferase IX, but in promyelocytes and monocytes by alpha 1,3-fucosyltransferase IV. J Biol Chem. 2001;276(19):16100-6.

60. Radonjic-Hoesli S, Valent P, Klion AD, Wechsler ME, Simon HU. Novel targeted therapies for eosinophil-associated diseases and allergy. Annu Rev Pharmacol Toxicol. 2015;55:633-56.

61. O'Sullivan JA, Bochner BS. Eosinophils and eosinophil-associated diseases: An update. J Allergy Clin Immunol. 2018;141(2):505-17.

62. Mishra A, Kuikel S, Rauniyar R, Poudel S, Thapa S, Pathak N, et al. An Unusual Case of Eosinophilia with Systemic Lupus Erythematosus: A Case Report and Review of Literature. Case Rep Med. 2022;2022:3264002.

63. Winterberg PD, Ford ML. The effect of chronic kidney disease on T cell alloimmunity. Current opinion in organ transplantation. 2017;22(1):22-8.

64. Meier P, Dayer E, Blanc E, Wauters JP. Early T cell activation correlates with expression of apoptosis markers in patients with end-stage renal disease. J Am Soc Nephrol. 2002;13(1):204-12.

65. Hartzell S, Bin S, Cantarelli C, Haverly M, Manrique J, Angeletti A, et al. Kidney Failure Associates With T Cell Exhaustion and Imbalanced Follicular Helper T Cells. Front Immunol. 2020;11:583702.

66. Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I. Disturbances of acquired immunity in hemodialysis patients. Seminars in dialysis. 2007;20(5):440-51.

67. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant. 2010;25(1):205-12.

68. Sendic S, Mansouri L, Lundberg S, Nopp A, Jacobson SH, Lundahl J. B cell and monocyte phenotyping: A quick asset to investigate the immune status in patients with IgA nephropathy. PLoS One. 2021;16(3):e0248056.

69. Smogorzewski M, Massry SG. Defects in B-cell function and metabolism in uremia: role of parathyroid hormone. Kidney Int Suppl. 2001;78:S186-9.

70. Guillevin L, Pagnoux C, Karras A, Khouatra C, Aumaître O, Cohen P, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med. 2014;371(19):1771-80.

71. Mejia-Vilet JM, Malvar A, Arazi A, Rovin BH. The Lupus Nephritis Management Renaissance. Kidney Int. 2021.

72. Lundberg S, Westergren E, Smolander J, Bruchfeld A. B cell-depleting therapy with rituximab or ofatumumab in immunoglobulin A nephropathy or vasculitis with nephritis. Clinical kidney journal. 2017;10(1):20-6.

73. KDIGO. Kidney Disease: Improving Global Outcomes (KDIGO) GD Work Group

KDIGO 2021 clinical practice guideline for the evaluation and management of chronic kidney disease

Kidney Int Suppl, will be published in the October issue of Supplements to Kidney International with an Executive Summary in the main pages of the journal.

74. Noto D, Miyake S. B cells in autoimmune diseases of the central nervous system. Clinical and Experimental Neuroimmunology. 2020;11(3):163-70.

75. Rahman A, Isenberg DA. Systemic lupus erythematosus. N Engl J Med. 2008;358(9):929-39.

76. Sarnak MJ, Jaber BL. Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. Kidney Int. 2000;58(4):1758-64.

77. Wang HE, Gamboa C, Warnock DG, Muntner P. Chronic kidney disease and risk of death from infection. Am J Nephrol. 2011;34(4):330-6.

78. Mansur A, Mulwande E, Steinau M, Bergmann I, Popov AF, Ghadimi M, et al. Chronic kidney disease is associated with a higher 90-day mortality than other chronic medical conditions in patients with sepsis. Sci Rep. 2015;5:10539.

79. Van Kerkhove MD, Vandemaele KA, Shinde V, Jaramillo-Gutierrez G, Koukounari A, Donnelly CA, et al. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. PLoS Med. 2011;8(7):e1001053.

80. Krueger KM, Ison MG, Ghossein C. Practical Guide to Vaccination in All Stages of CKD, Including Patients Treated by Dialysis or Kidney Transplantation. Am J Kidney Dis. 2020;75(3):417-25.

81. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. Science. 2022;375(6585):1122-7.

82. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020;584(7821):430-6.

83. Flythe JE, Assimon MM, Tugman MJ, Chang EH, Gupta S, Shah J, et al. Characteristics and Outcomes of Individuals With Pre-existing Kidney Disease and COVID-19 Admitted to Intensive Care Units in the United States. Am J Kidney Dis. 2020.

84. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2021;93(1):250-6.

85. Hussman JP. Cellular and Molecular Pathways of COVID-19 and Potential Points of Therapeutic Intervention. Front Pharmacol. 2020;11:1169.

86. Shive CL, Jiang W, Anthony DD, Lederman MM. Soluble CD14 is a nonspecific marker of monocyte activation. AIDS. 2015;29(10):1263-5.

87. Gómez-Rial J, Currás-Tuala MJ, Rivero-Calle I, Gómez-Carballa A, Cebey-López M, Rodríguez-Tenreiro C, et al. Increased Serum Levels of sCD14 and sCD163 Indicate a Preponderant Role for Monocytes in COVID-19 Immunopathology. Front Immunol. 2020;11:560381.

88. Ramasamy S, Subbian S. Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis. Clin Microbiol Rev. 2021;34(3).

89. Singh S, Anshita D, Ravichandiran V. MCP-1: Function, regulation, and involvement in disease. Int Immunopharmacol. 2021;101(Pt B):107598.

90. Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. Cytokine Growth Factor Rev. 2002;13(6):455-81.

91. Chen Y, Wang J, Liu C, Su L, Zhang D, Fan J, et al. IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. Mol Med. 2020;26(1):97.

92. Coppo R. Treatment of IgA nephropathy: Recent advances and prospects. Nephrol Ther. 2018;14 Suppl 1:S13-s21.

93. D'Amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. Semin Nephrol. 2004;24(3):179-96.

94. Coppo R. Clinical and histological risk factors for progression of IgA nephropathy: an update in children, young and adult patients. Journal of nephrology. 2017;30(3):339-46.

95. Barratt J, Feehally J. IgA nephropathy. J Am Soc Nephrol. 2005;16(7):2088-97.

96. Rizk DV, Maillard N, Julian BA, Knoppova B, Green TJ, Novak J, et al. The Emerging Role of Complement Proteins as a Target for Therapy of IgA Nephropathy. Front Immunol. 2019;10:504.

97. Maixnerová D, Tesař V, Ryšavá R, Reiterová J, Poupětová H, Dvořáková L, et al. The coincidence of IgA nephropathy and Fabry disease. BMC Nephrol. 2013;14:6.

98. Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, et al. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. Kidney Int. 2009;76(5):534-45.

99. Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, et al. Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. Kidney Int. 2017;91(5):1014-21.

100. Boyd JK, Cheung CK, Molyneux K, Feehally J, Barratt J. An update on the pathogenesis and treatment of IgA nephropathy. Kidney Int. 2012;81(9):833-43.

101. Lai KN. Pathogenesis of IgA nephropathy. Nature reviews Nephrology. 2012;8(5):275-83.

102. Magistroni R, D'Agati VD, Appel GB, Kiryluk K. New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. Kidney Int. 2015;88(5):974-89.

103. Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, et al. The pathophysiology of IgA nephropathy. J Am Soc Nephrol. 2011;22(10):1795-803.

104. Lai KN, Tang SC, Schena FP, Novak J, Tomino Y, Fogo AB, et al. IgA nephropathy. Nature reviews Disease primers. 2016;2:16001.

105. Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, Lifton RP, et al. Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol. 2008;19(5):1008-14.

106. Kiryluk K, Novak J. The genetics and immunobiology of IgA nephropathy. J Clin Invest. 2014;124(6):2325-32.

107. Plomp R, Dekkers G, Rombouts Y, Visser R, Koeleman CA, Kammeijer GS, et al. Hinge-Region O-Glycosylation of Human Immunoglobulin G3 (IgG3). Mol Cell Proteomics. 2015;14(5):1373-84.

108. Smith AC, de Wolff JF, Molyneux K, Feehally J, Barratt J. O-glycosylation of serum IgD in IgA nephropathy. J Am Soc Nephrol. 2006;17(4):1192-9.

109. Yanagawa H, Suzuki H, Suzuki Y, Kiryluk K, Gharavi AG, Matsuoka K, et al. A panel of serum biomarkers differentiates IgA nephropathy from other renal diseases. PLoS One. 2014;9(5):e98081.

110. Maixnerova D, Tesar V. Emerging role of monoclonal antibodies in the treatment of IgA nephropathy. Expert Opin Biol Ther. 2023;23(5):419-27.

111. Floege J, Rauen T, Tang SCW. Current treatment of IgA nephropathy. Semin Immunopathol. 2021.

112. Lafayette RA, Canetta PA, Rovin BH, Appel GB, Novak J, Nath KA, et al. A Randomized, Controlled Trial of Rituximab in IgA Nephropathy with Proteinuria and Renal Dysfunction. J Am Soc Nephrol. 2017;28(4):1306-13.

113. Barratt J, Hour B., Kooienga L., Roy S., Schwartz B., Siddiqui A., Tolentino J., IYER S.P., Stromatt C., Endsley A., Lo J., Glicklich A. . POS-109 Interim results of phase 1 and 2 trials to investigate the safety, tolerability, pharmacokinetics, pharmacodynamics, and clinical activity of BION-1301 in patients with IgA Nephropathy Kidney international reports. 2022;7(2):S46.

114. Barratt J, Tumlin J, Suzuki Y, Kao A, Aydemir A, Pudota K, et al. Randomized Phase II JANUS Study of Atacicept in Patients With IgA Nephropathy and Persistent Proteinuria. Kidney international reports. 2022;7(8):1831-41.

115. Lv J, Liu L, Hao C, Li G, Fu P, Xing G, et al. Randomized Phase 2 Trial of Telitacicept in Patients With IgA Nephropathy With Persistent Proteinuria. Kidney international reports. 2023;8(3):499-506.

116. Petri M, Buyon J, Kim M. Classification and definition of major flares in SLE clinical trials. Lupus. 1999;8(8):685-91.

117. Parikh SV, Almaani S, Brodsky S, Rovin BH. Update on Lupus Nephritis: Core Curriculum 2020. Am J Kidney Dis. 2020;76(2):265-81.

118. Markowitz GS, D'Agati VD. The ISN/RPS 2003 classification of lupus nephritis: an assessment at 3 years. Kidney Int. 2007;71(6):491-5.

119. Almaani S, Meara A, Rovin BH. Update on Lupus Nephritis. Clin J Am Soc Nephrol. 2017;12(5):825-35.

120. Bengtsson AA, Rönnblom L. Systemic lupus erythematosus: still a challenge for physicians. J Intern Med. 2017;281(1):52-64.

121. Charles N, Rivera J. Basophils and autoreactive IgE in the pathogenesis of systemic lupus erythematosus. Curr Allergy Asthma Rep. 2011;11(5):378-87.

122. Sawalha AH, Harley JB. Antinuclear autoantibodies in systemic lupus erythematosus. Curr Opin Rheumatol. 2004;16(5):534-40.

123. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110-21.

124. Dema B, Pellefigues C, Hasni S, Gault N, Jiang C, Ricks TK, et al. Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. PLoS One. 2014;9(2):e90424.

125. Dema B, Charles N, Pellefigues C, Ricks TK, Suzuki R, Jiang C, et al. Immunoglobulin E plays an immunoregulatory role in lupus. J Exp Med. 2014;211(11):2159-68.

126. Menzella F, Just J, Sauerbeck IS, Mailaender C, Saccheri F, Thonnelier C, et al. Omalizumab for the treatment of patients with severe allergic asthma with immunoglobulin E levels above >1500 IU/mL. The World Allergy Organization journal. 2023;16(6):100787.

127. Maurer M, Rosén K, Hsieh HJ, Saini S, Grattan C, Gimenéz-Arnau A, et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. N Engl J Med. 2013;368(10):924-35.

128. Hasni S, Gupta S, Davis M, Poncio E, Temesgen-Oyelakin Y, Joyal E, et al. Safety and Tolerability of Omalizumab: A Randomized Clinical Trial of Humanized Anti-IgE Monoclonal Antibody in Systemic Lupus Erythematosus. Arthritis & rheumatology (Hoboken, NJ). 2019;71(7):1135-40.

129. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options. Circulation. 2021;143(11):1157-72.

130. Chen J, Budoff MJ, Reilly MP, Yang W, Rosas SE, Rahman M, et al. Coronary Artery Calcification and Risk of Cardiovascular Disease and Death Among Patients With Chronic Kidney Disease. JAMA cardiology. 2017;2(6):635-43.

131. Hutcheson JD, Goettsch C. Cardiovascular Calcification Heterogeneity in Chronic Kidney Disease. Circ Res. 2023;132(8):993-1012.

132. Madero M, Peralta C, Katz R, Canada R, Fried L, Najjar S, et al. Association of arterial rigidity with incident kidney disease and kidney function decline: the Health ABC study. Clin J Am Soc Nephrol. 2013;8(3):424-33.

133. Ford ML, Tomlinson LA, Chapman TP, Rajkumar C, Holt SG. Aortic stiffness is independently associated with rate of renal function decline in chronic kidney disease stages 3 and 4. Hypertension. 2010;55(5):1110-5.

134. Dai L, Qureshi AR, Witasp A, Lindholm B, Stenvinkel P. Early Vascular Ageing and Cellular Senescence in Chronic Kidney Disease. Computational and structural biotechnology journal. 2019;17:721-9.

135. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med. 2000;342(20):1478-83.

136. O'Hare AM, Glidden DV, Fox CS, Hsu CY. High prevalence of peripheral arterial disease in persons with renal insufficiency: results from the National Health and Nutrition Examination Survey 1999-2000. Circulation. 2004;109(3):320-3.

137. Arroyo D, Betriu A, Valls J, Gorriz JL, Pallares V, Abajo M, et al. Factors influencing pathological ankle-brachial index values along the chronic kidney disease spectrum: the NEFRONA study. Nephrol Dial Transplant. 2017;32(3):513-20.

138. Gisterå A, Hansson GK. The immunology of atherosclerosis. Nature reviews Nephrology. 2017;13(6):368-80.

139. Hénaut L, Candellier A, Boudot C, Grissi M, Mentaverri R, Choukroun G, et al. New Insights into the Roles of Monocytes/Macrophages in Cardiovascular Calcification Associated with Chronic Kidney Disease. Toxins (Basel). 2019;11(9).

140. Shanahan CM. Mechanisms of vascular calcification in CKD-evidence for premature ageing? Nature reviews Nephrology. 2013;9(11):661-70.

141. Lee SJ, Lee IK, Jeon JH. Vascular Calcification-New Insights Into Its Mechanism. Int J Mol Sci. 2020;21(8).

142. Shanahan CM, Crouthamel MH, Kapustin A, Giachelli CM. Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res. 2011;109(6):697-711.

143. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008;473(2):139-46.

144. Rochette L, Meloux A, Rigal E, Zeller M, Malka G, Cottin Y, et al. The Role of Osteoprotegerin in Vascular Calcification and Bone Metabolism: The Basis for Developing New Therapeutics. Calcif Tissue Int. 2019;105(3):239-51.

145. Dutka M, Bobiński R, Wojakowski W, Francuz T, Pająk C, Zimmer K. Osteoprotegerin and RANKL-RANK-OPG-TRAIL signalling axis in heart failure and other cardiovascular diseases. Heart Fail Rev. 2021.

146. Harper E, Forde H, Davenport C, Rochfort KD, Smith D, Cummins PM. Vascular calcification in type-2 diabetes and cardiovascular disease: Integrative roles for OPG, RANKL and TRAIL. Vascul Pharmacol. 2016;82:30-40.

147. Abedin M, Omland T, Ueland T, Khera A, Aukrust P, Murphy SA, et al. Relation of osteoprotegerin to coronary calcium and aortic plaque (from the Dallas Heart Study). Am J Cardiol. 2007;99(4):513-8.

148. Ziegler S, Kudlacek S, Luger A, Minar E. Osteoprotegerin plasma concentrations correlate with severity of peripheral artery disease. Atherosclerosis. 2005;182(1):175-80.

149. Dekker M, Waissi F, Silvis MJM, Bennekom JV, Schoneveld AH, de Winter RJ, et al. High levels of osteoprotegerin are associated with coronary artery calcification in patients suspected of a chronic coronary syndrome. Sci Rep. 2021;11(1):18946.

150. Pedersen S, Mogelvang R, Bjerre M, Frystyk J, Flyvbjerg A, Galatius S, et al. Osteoprotegerin predicts long-term outcome in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. Cardiology. 2012;123(1):31-8.

151. Reinhard H, Nybo M, Hansen PR, Wiinberg N, Kjær A, Petersen CL, et al. Osteoprotegerin and coronary artery disease in type 2 diabetic patients with microalbuminuria. Cardiovasc Diabetol. 2011;10:70.

152. Jorsal A, Tarnow L, Flyvbjerg A, Parving HH, Rossing P, Rasmussen LM. Plasma osteoprotegerin levels predict cardiovascular and all-cause mortality and deterioration of kidney function in type 1 diabetic patients with nephropathy. Diabetologia. 2008;51(11):2100-7.

153. Nitta K, Akiba T, Uchida K, Otsubo S, Takei T, Yumura W, et al. Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis patients. Nephrol Dial Transplant. 2004;19(7):1886-9.

154. Ozkok A, Caliskan Y, Sakaci T, Erten G, Karahan G, Ozel A, et al. Osteoprotegerin/RANKL axis and progression of coronary artery calcification in hemodialysis patients. Clin J Am Soc Nephrol. 2012;7(6):965-73.

155. Kamińska J, Stopiński M, Mucha K, Pac M, Gołębiowski M, Niewczas MA, et al. Circulating Osteoprotegerin in Chronic Kidney Disease and All-Cause Mortality. Int J Gen Med. 2021;14:2413-20.

156. Cucci LM, Satriano C, Marzo T, La Mendola D. Angiogenin and Copper Crossing in Wound Healing. Int J Mol Sci. 2021;22(19).

157. Yu P, Liu M, Yang X, Yu Y, Zhao J, Zhang L, et al. Diagnostic Utility of ANG in Coronary Heart Disease Complicating Chronic Heart Failure: A Cross-Sectional Study. Dis Markers. 2016;2016:2740826.

158. Choi HM, Kwon YE, Kim S, Oh DJ. Changes in FGF-23, Neutrophil/Platelet Activation Markers, and Angiogenin in Advanced Chronic Kidney Disease and Their Effect on Arterial Stiffness. Kidney Blood Press Res. 2019;44(5):1166-78.

159. Höbaus C, Pesau G, Zierfuss B, Koppensteiner R, Schernthaner GH. Angiogenin-A Proposed Biomarker for Cardiovascular Disease-Is Not Associated With Long-Term Survival in Patients With Peripheral Artery Disease. Angiology. 2021;72(9):855-60.

160. Kamath TP, Prasad R, Allison MA, Criqui MC, Ix JH, Rifkin DE, et al. Association of Ankle-Brachial and Toe-Brachial Indexes With Mortality in Patients With CKD. Kidney medicine. 2020;2(1):68-75.

161. Dorans KS, He H, Chen J, Dobre M, Go AS, Hamm LL, et al. Change in ankle-brachial index and mortality among individuals with chronic kidney disease: findings from the Chronic Renal Insufficiency Cohort Study. Nephrol Dial Transplant. 2021;36(12):2224-31.

162. Kousios A, Kouis P, Hadjivasilis A, Panayiotou A. Cardiovascular Risk Assessment Using Ultrasonographic Surrogate Markers of Atherosclerosis and Arterial Stiffness in Patients With Chronic Renal Impairment: A Narrative Review of the Evidence and a Critical View of Their Utility in Clinical Practice. Canadian journal of kidney health and disease. 2020;7:2054358120954939.

163. Karras A, Haymann JP, Bozec E, Metzger M, Jacquot C, Maruani G, et al. Large artery stiffening and remodeling are independently associated with all-cause mortality and cardiovascular events in chronic kidney disease. Hypertension. 2012;60(6):1451-7.

164. Svenungsson E, Engelbertsen D, Wigren M, Gustafsson JT, Gunnarsson I, Elvin K, et al. Decreased levels of autoantibodies against apolipoprotein B-100 antigens are associated with cardiovascular disease in systemic lupus erythematosus. Clin Exp Immunol. 2015;181(3):417-26.

165. McKinnon KM. Flow Cytometry: An Overview. Curr Protoc Immunol. 2018;120:5.1.-5.1.11.

166. Shah H, Eisenbarth S, Tormey CA, Siddon AJ. Behind the scenes with basophils: an emerging therapeutic target. Immunotherapy advances. 2021;1(1):ltab008.

167. Cromheecke JL, Nguyen KT, Huston DP. Emerging role of human basophil biology in health and disease. Curr Allergy Asthma Rep. 2014;14(1):408.

168. Yuan J, Gou SJ, Huang J, Hao J, Chen M, Zhao MH. C5a and its receptors in human anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Arthritis Res Ther. 2012;14(3):R140.

169. Wang Y, Zhang H, He YW. The Complement Receptors C3aR and C5aR Are a New Class of Immune Checkpoint Receptor in Cancer Immunotherapy. Front Immunol. 2019;10:1574.

170. Wolanczyk-Medrala A, Barg W, Medrala W. CD164 as a Basophil Activation Marker. Curr Pharm Des. 2011;17(34):3786-96.

171. Katayama Y, Hidalgo A, Chang J, Peired A, Frenette PS. CD44 is a physiological E-selectin ligand on neutrophils. J Exp Med. 2005;201(8):1183-9.

172. Grozdanovic M, Laffey KG, Abdelkarim H, Hitchinson B, Harijith A, Moon HG, et al. Novel peptide nanoparticle-biased antagonist of CCR3 blocks eosinophil recruitment and airway hyperresponsiveness. J Allergy Clin Immunol. 2019;143(2):669-80.e12.

173. Mountjoy KG. ELISA versus LUMINEX assay for measuring mouse metabolic hormones and cytokines: sharing the lessons I have learned. J Immunoassay Immunochem. 2021;42(2):154-73.

174. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.

175. Ayoglu B, Chaouch A, Lochmüller H, Politano L, Bertini E, Spitali P, et al. Affinity proteomics within rare diseases: a BIO-NMD study for blood biomarkers of muscular dystrophies. EMBO Mol Med. 2014;6(7):918-36.

176. Drobin K, Nilsson P, Schwenk JM. Highly multiplexed antibody suspension bead arrays for plasma protein profiling. Methods Mol Biol. 2013;1023:137-45.

177. Neiman M, Hedberg JJ, Dönnes PR, Schuppe-Koistinen I, Hanschke S, Schindler R, et al. Plasma profiling reveals human fibulin-1 as candidate marker for renal impairment. J Proteome Res. 2011;10(11):4925-34.

178. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.

179. Sallustio F, Curci C, Chaoul N, Fontò G, Lauriero G, Picerno A, et al. High levels of gut-homing immunoglobulin A+ B lymphocytes support the pathogenic role of intestinal mucosal

hyperresponsiveness in immunoglobulin A nephropathy patients. Nephrol Dial Transplant. 2021;36(3):452-64.

180. He JW, Zhou XJ, Lv JC, Zhang H. Perspectives on how mucosal immune responses, infections and gut microbiome shape IgA nephropathy and future therapies. Theranostics. 2020;10(25):11462-78.

181. Gesualdo L, Di Leo V, Coppo R. The mucosal immune system and IgA nephropathy. Semin Immunopathol. 2021;43(5):657-68.

182. Ruszkowski J, Lisowska KA, Pindel M, Heleniak Z, Debska-Slizien A, Witkowski JM. T cells in IgA nephropathy: role in pathogenesis, clinical significance and potential therapeutic target. Clin Exp Nephrol. 2019;23(3):291-303.

183. Tang Y, He H, Hu P, Xu X. T lymphocytes in IgA nephropathy. Exp Ther Med. 2020;20(1):186-94.

184. Mukherjee R, Kanti Barman P, Kumar Thatoi P, Tripathy R, Kumar Das B, Ravindran B. Non-Classical monocytes display inflammatory features: Validation in Sepsis and Systemic Lupus Erythematous. Sci Rep. 2015;5:13886.

185. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008;112(5):1570-80.

186. Contin C, Pitard V, Delmas Y, Pelletier N, Defrance T, Moreau JF, et al. Potential role of soluble CD40 in the humoral immune response impairment of uraemic patients. Immunology. 2003;110(1):131-40.

187. Tapia-Llanos R, Muñoz-Valle JF, Román-Fernández IV, Marín-Rosales M, Salazar-Camarena DC, Cruz A, et al. Association of soluble CD40 levels with -1 C > T CD40 polymorphism and chronic kidney disease in systemic lupus erythematosus. Molecular genetics & genomic medicine. 2019;7(12):e1014.

188. Tanaka K, Sugiyama H, Morinaga H, Kitagawa M, Kano Y, Onishi Y, et al. Serum sCD40L and IL-31 in Association with Early Phase of IgA Nephropathy. Journal of clinical medicine. 2023;12(5).

189. Diehl S, Rincón M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. 2002;39(9):531-6.

190. Ohsaki Y, Takahashi S, Scarcez T, Demulder A, Nishihara T, Williams R, et al. Evidence for an autocrine/paracrine role for interleukin-6 in bone resorption by giant cells from giant cell tumors of bone. Endocrinology. 1992;131(5):2229-34.

191. Chavele KM, Merry E, Ehrenstein MR. Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. J Immunol. 2015;194(6):2482-5.

192. Moser K, Muehlinghaus G, Manz R, Mei H, Voigt C, Yoshida T, et al. Long-lived plasma cells in immunity and immunopathology. Immunol Lett. 2006;103(2):83-5.

193. Vianna HR, Soares CM, Silveira KD, Elmiro GS, Mendes PM, de Sousa Tavares M, et al. Cytokines in chronic kidney disease: potential link of MCP-1 and dyslipidemia in glomerular diseases. Pediatr Nephrol. 2013;28(3):463-9.

194. Morii T, Fujita H, Narita T, Shimotomai T, Fujishima H, Yoshioka N, et al. Association of monocyte chemoattractant protein-1 with renal tubular damage in diabetic nephropathy. J Diabetes Complications. 2003;17(1):11-5.

195. Scurt FG, Menne J, Brandt S, Bernhardt A, Mertens PR, Haller H, et al. Monocyte chemoattractant protein-1 predicts the development of diabetic nephropathy. Diabetes Metab Res Rev. 2022;38(2):e3497.

196. Si R, Zhao P, Yu Z, Qu Z, Sun W, Li T, et al. Increased Non-switched Memory B Cells are Associated with Plasmablasts, Serum IL-6 Levels and Renal Functional Impairments in IgAN Patients. Immunol Invest. 2019:1-13.

197. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012;12(3):191-200.

198. Kudose S, Santoriello D, Bomback AS, Stokes MB, D'Agati VD, Markowitz GS. Sensitivity and Specificity of Pathologic Findings to Diagnose Lupus Nephritis. Clin J Am Soc Nephrol. 2019;14(11):1605-15.

199. Davidson A. What is damaging the kidney in lupus nephritis? Nat Rev Rheumatol. 2016;12(3):143-53.

200. Fujimoto S, Arinobu Y, Miyawaki K, Ayano M, Mitoma H, Kimoto Y, et al. Anti-dsDNA IgE induces IL-4 production from basophils, potentially involved in B-cell differentiation in systemic lupus erythematosus. Rheumatology (Oxford). 2023.

201. Pellefigues C, Dema B, Lamri Y, Saidoune F, Chavarot N, Lohéac C, et al. Prostaglandin D(2) amplifies lupus disease through basophil accumulation in lymphoid organs. Nature communications. 2018;9(1):725.

202. Gorabi AM, Hajighasemi S, Kiaie N, Gheibi Hayat SM, Jamialahmadi T, Johnston TP, et al. The pivotal role of CD69 in autoimmunity. J Autoimmun. 2020;111:102453.

203. Ssemaganda A, Kindinger L, Bergin P, Nielsen L, Mpendo J, Ssetaala A, et al. Characterization of neutrophil subsets in healthy human pregnancies. PLoS One. 2014;9(2):e85696.

204. Cairns AP, Crockard AD, McConnell JR, Courtney PA, Bell AL. Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: relations with apoptotic neutrophils and disease activity. Ann Rheum Dis. 2001;60(10):950-5.

205. Tsokos GC, Lo MS, Costa Reis P, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol. 2016;12(12):716-30.

206. Hellmark T, Ohlsson S, Pettersson Å, Hansson M, Johansson Å CM. Eosinophils in anti-neutrophil cytoplasmic antibody associated vasculitis. BMC rheumatology. 2019;3:9.

207. Chuang HC, Chen MH, Chen YM, Ciou YR, Hsueh CH, Tsai CY, et al. Induction of Interferon-γ and Tissue Inflammation by Overexpression of Eosinophil Cationic Protein in T Cells and Exosomes. Arthritis & rheumatology (Hoboken, NJ). 2022;74(1):92-104.

208. Johansen KL, Garimella PS, Hicks CW, Kalra PA, Kelly DM, Martens S, et al. Central and peripheral arterial diseases in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2021;100(1):35-48.

209. Nie F, He J, Cao H, Hu X. Predictive value of abnormal ankle-brachial index in patients with diabetes: A meta-analysis. Diabetes Res Clin Pract. 2021;174:108723.

210. Smith FB, Lee AJ, Price JF, van Wijk MC, Fowkes FG. Changes in ankle brachial index in symptomatic and asymptomatic subjects in the general population. J Vasc Surg. 2003;38(6):1323-30.

211. Gu X, Man C, Zhang H, Fan Y. High ankle-brachial index and risk of cardiovascular or all-cause mortality: A meta-analysis. Atherosclerosis. 2019;282:29-36.

212. Kim SM, Lee J, Ryu OH, Lee KW, Kim HY, Seo JA, et al. Serum osteoprotegerin levels are associated with inflammation and pulse wave velocity. Clin Endocrinol (Oxf). 2005;63(5):594-8.

213. Amar J, Ruidavets JB, Bal Dit Sollier C, Bongard V, Boccalon H, Chamontin B, et al. Soluble CD14 and aortic stiffness in a population-based study. J Hypertens. 2003;21(10):1869-77.

214. Asp AM, Wallquist C, Rickenlund A, Hylander B, Jacobson SH, Caidahl K, et al. Aspects of carotid structure and function in health and different stages of chronic kidney disease. Clin Physiol Funct Imaging. 2018;38(3):402-8.

215. Morisawa T, Nakagomi A, Kohashi K, Kosugi M, Kusama Y, Atarashi H, et al. Osteoprotegerin is Associated With Endothelial Function and Predicts Early Carotid Atherosclerosis in Patients With Coronary Artery Disease. Int Heart J. 2015;56(6):605-12.

216. Vik A, Mathiesen EB, Brox J, Wilsgaard T, Njølstad I, Jørgensen L, et al. Relation between serum osteoprotegerin and carotid intima media thickness in a general population - the Tromsø Study. J Thromb Haemost. 2010;8(10):2133-9.

217. Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis. 2009;204(2):321-9.

218. Tsuyuki K, Kohno K, Ebine K, Obara T, Aoki T, Muto A, et al. Exercise-ankle brachial pressure index with one-minute treadmill walking in patients on maintenance hemodialysis. Annals of vascular diseases. 2013;6(1):52-6.

219. Kouis P, Kousios A, Kanari A, Kleopa D, Papatheodorou SI, Panayiotou AG. Association of non-invasive measures of subclinical atherosclerosis and arterial stiffness with mortality and major cardiovascular events in chronic kidney disease: systematic review and meta-analysis of cohort studies. Clinical kidney journal. 2020;13(5):842-54.

220. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol. 2015;15(3):160-71.

221. Halliley JL, Tipton CM, Liesveld J, Rosenberg AF, Darce J, Gregoretti IV, et al. Long-Lived Plasma Cells Are Contained within the CD19(-)CD38(hi)CD138(+) Subset in Human Bone Marrow. Immunity. 2015;43(1):132-45.

222. Kalm F, Mansouri L, Russom A, Lundahl J, Nopp A. Adhesion molecule cross-linking and cytokine exposure modulate IgE- and non-IgE-dependent basophil activation. Immunology. 2021;162(1):92-104.